(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/36003 A2

(51) International Patent Classification[7]: A61K 47/48

(21) International Application Number: PCT/US00/31262

(22) International Filing Date: 14 November 2000 (14.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/165,485	15 November 1999 (15.11.1999)	US
60/239,478	11 October 2000 (11.10.2000)	US
60/241,939	20 October 2000 (20.10.2000)	US

(71) Applicant *(for all designated States except US)*: DRUG INNOVATION & DESIGN, INC. [US/US]; 415 South Street, Waltham, MA 02254 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: GLAZIER, Arnold [US/US]; 9 Brandeis Road, Newton, MA 02159 (US).

(74) Agents: PIERCE, N. Scott et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/36003 A2

(54) Title: SELECTIVE CELLULAR TARGETING: MULTIFUNCTIONAL DELIVERY VEHICLES

(57) Abstract: The present invention relates to the compositions, methods, and applications of a novel approach to selective cellular targeting. The purpose of this invention is to enable the selective delivery and/or selective activation of effector molecules to target cells for diagnostic or therapeutic purposes. The present invention relates to multi-functional prodrugs or targeting vehicles wherein each functionality is capable of enhancing targeting selectivity, affinity, intracellular transport, activation or detoxification. The present invention also relates to ultralow dose, multiple target, multiple drug chemotherapy and targeted immunotherapy for cancer treatment.

Selective Cellular Targeting: Multifunctional Delivery Vehicles

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 60/165,485, filed on November 15,1999, U.S. Provisional Application Serial No. 60/239,478, filed on October 11, 2000, and U.S. Provisional Application Serial No. 60/241,939, filed on October 20,2000. The entire teachings of these applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION:

Approximately 8 million Americans have a history of cancer. An estimated 500,000 people in the U.S. die from cancer yearly. The need for new and improved anti-cancer drugs is clear and compelling. The goal of cancer chemotherapy is to kill all malignant cells without undo toxicity to the patient. The fundamental technical obstacle to the development of safe and effective anti-cancer drugs is the problem of tumor selectivity. Cells become malignant by the abnormal regulation of normal cellular functions caused by changes in DNA. With few exceptions the quest for an enzyme or target which is *absolutely* selective for malignant cells has been elusive. Furthermore, it has become increasingly evident that an enormous number of gene defects that interfere with the regulation of cell growth and proliferation can cause cancer or reinforce the malignant state.

Hard learned lessons in pediatric oncology have defined the clinical requirements for the complete eradication of cancer. The administration of multiple drugs each capable of independently giving a 1-3 log reduction of tumor burden without the combined drug toxicity producing unacceptable side effects. The following reference relates to this subject matter: Frei, E. III., "Curative Cancer Chemotherapy," *Cancer Res,* 45(12 Pt 1):6523-37 (1985), the contents of which are incorporated herein by reference in their entirety. Drug toxicity due to the low selectivity of anti-cancer drugs is the fundamental barrier to the routine cure of cancer. A compounding factor is the development of drug resistance. Current therapeutic regimens attempt to deal with the problem of drug resistance by the administration of multiple agents. However, the combined

toxicity of multiple agents limits the effectiveness of this approach. Enormous efforts have been directed to the development of highly selective anti-cancer drugs. Monoclonal antibodies have been employed as targeting agents for the delivery of cytotoxic drugs to tumors. However, very few antigens, that are *absolutely* tumor specific, are available for tumor targeting. In addition monoclonal antibodies are large molecules, and often do not penetrate well into tumors. Proteins and oligopeptides have also been used as targeting agents. Small molecules described as targeting agents include: folate, sigma receptor binding agents and agmatine. A variety of approaches have also been described to target cells by prodrugs, which are activated by enzymes that are increased in tumor cells. Despite great efforts a general solution to the problem of selective cell targeting and selective destruction of cancer cells remains elusive. This is the subject of the present invention..

SUMMARY OR THE INVENTION:

The present invention relates to the compositions, methods, and applications of a novel approach to selective cellular targeting. The purpose of this invention is to enable the selective delivery and/or selective activation of effector molecules to target cells for diagnostic or therapeutic purposes. The present invention relates to multi-functional prodrugs or targeting vehicles wherein each functionality is capable of enhancing targeting selectivity, affinity, intracellular transport, activation or detoxification. The present invention also relates to ultra-low dose, multiple target, multiple drug chemotherapy and targeted immunotherapy for cancer treatment.

BREIF DESCRIPTION OF THE DRAWINGS:

No drawings

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

Analog - A compound or moiety possessing significant structural similarity as to possess substantially the same function.

At a Target Cell - A phrase used to refer to in, on, or in the microenvironment of a target cell.

Binding Affinity - Tightness of binding between a ligand receptor.

Bioreversibly Masked Group - A chemical group that is derivatized in a bioreversible manner. For example, an ester group can be a bioreversibly masked group for a hydroxy group. A bioreversible masking group is a chemical group that when bonded with a second group produces a bioreversibly masked group for said second group.

Bioreversible Protecting Group - A chemical group or trigger that can be modified in vivo and wherein said modification unmasks the group which is protected.

Chemically Modify - To change the chemical property of a molecule by making one or more new chemical bonds and/or by breaking one or more chemical bonds of the molecule.

Connectivity - The sites at which chemical structures or functional groups are attached together to give a single molecule. For example, various connectivity between groups A, B, C include structures such as A-B-C,

B-A-C, or A-C-B. Connectivity can be direct such as by a covalent bond between an atom of A and B or indirect such as through a covalently bonded linker.

Derivative - A compound or moiety that has been further modified or functionalized from the corresponding compound or moiety.

Effector – An agent that exerts an activity and evokes a physical, chemical or biological response such as a pharmacologically beneficial response such as cytotoxicity, or a diagnostic effect.

Functional Cooperation between Components - If the effect produced by two or more components of a drug acting jointly or together is greater than the effect produced by the components acting individually or independently the components "functionally cooperate".

Good Leaving Group - A chemical group that readily cleaves from the group to which it is attached. For example, a group that is easily displaced in a nucleophilic reaction, or which undergoes facile solvolysis in an SN1 type reaction.

Inert Substituents - A chemical substituent which does not interfere with functionality to a significant degree.

Linker – A chemical group that serves to attach targeting ligands, triggers and effectors or other chemical structures together.

Lower Alkyl Group - A hydrocarbon containing about 10 or less carbon atoms which can be linear or cyclic and which can bear substituents.

Masked Group - A chemical group that is hidden or blocked,or derivatized until unmasked.

Microenvironment of the target - The volume of space around a target cell within which a drug is able to evoke its intended pharmacological activity upon the target. Alternatively, the volume encompassed by a sphere centered on a tumor cell with a radius of between about 10 to about 500 microns.

Multifactorial - A function of multiple factors or variables.

Multivalent Binding- Binding at multiple targeting ligand- target receptor sites.

Non-selective Targeting Ligand- A chemical structure that binds to a receptor or physically associates with biomolecules that are ubiquitous or not enriched on the target compared to non-target.

Non-target- A cell, cells, tissue, or tissue type to which it is not desired to direct effector activity, such as normal cells, bone marrow stem cells, or normal liver.

Over-expressed- present at increased amounts.

Pharmacological activity- A beneficial physical, chemical or biological response that is evoked by a drug or effector agent such as a cytotoxicity or stimulation of the immune system or a diagnostic effect.

Target- A cell, cells, tissue, or tissue type to which it is desired to direct effector activity such as tumor cells, or autoimmune lymphocytes.

Targeting Agent- A chemical structure or group of chemical structures composed of targeting ligand(s) and/or trigger(s) that confer a degree of specificity towards a target.

Targeting Ligand – A chemical structure, which binds with a degree of specificity to a targeting receptor that is enriched at a target cell compared to at a non-target cell.

Targeting Property- Any characteristic, feature, or factor, such as a targeting receptor, a triggering agent, an enzyme, or a chemical or biochemical factor that can be used to distinguish between target and non-target.

Targeting Receptor- A chemical structure at the target that binds with a useful degree of specificity to a targeting ligand that is present in increased amounts in a target compared to a non-target but not necessarily all non-targets.

Targeting Selectivity- The ability to evoke a greater effector activity at target compared to non-target.

Target Molecules- Biomolecules that are either target receptors or triggering agents such as a protein that binds a targeting ligand or an enzyme at the target cell which can activate a trigger and which are increased at a target compared to a non-target but not necessarily all non-targets.

Trigger- A chemical group which can undergo in vivo chemical modification either spontaneously or by a triggering agent with the modification leading to trigger activation that modulates the pharmacological activity of the drug. A trigger can be considered as a chemical switch that upon activation gives a consistent and predictable output such as unmasking a chemical group, or detoxifying the drug, or toxifying the drug, or liberating an effector agent.

Trigger Activation- The process of chemical modification that causes a trigger to modulate the pharmacological activity of the drug.

Triggering Factor- An enzyme, biomolecule or other agent which is able to activate a trigger, also referred to as a "triggering agent".

Tumor Component - is a biomolecule which is present in tumor cells, on tumor cells, in the microenvironment of tumor cells, on tumor stromal cells or present in tumor bulk.

Tumor-selective Target Receptor – A target receptor that is present in increased amounts on tumor cells or in the microenvironment of tumor cells compared to that of normal cells but not necessarily all types of normal cells.

Tumor-selective Triggering Agent – A triggering agent or triggering factor that is present in increased amounts on tumor cells, in tumor cells, or in the microenvironment of tumor cells compared to that of normal cells but not necessarilly all types of normal cells.

Vital Normal Cells- Cells that if destroyed would produce unacceptable clinical toxicity to a patient such as bone marrow stem cells, liver cells and cardiac cells.

In order to eradicate cancer it is necessary to administer sufficient drugs to kill the last cancer cell without prohibitive toxicity to the patient. The poor selectivity and high toxicity of current anti-cancer drugs is the major road-block to routinely achieving this goal. What is needed is a technology that can allow the safe use of multiple drugs directed against multiple properties of the tumor without multiple toxicity. This invention relates to an integrated description of technologies directed towards this goal.

There are two fundamental problems in anti-cancer drug design and therapy:

1.) Absolute enzymatic differences between normal and malignant cells are with rare exceptions elusive.

2.) Tumors are heterogenous and can develop resistance to any drug.

In order for any type of therapy to selectively kill cancer cells the therapy must be directed to differences between normal cells and cancer cells.

There are two types of differences:

1.) Specific differences that are the causative lesions of cancer.

2.) Nonspecific differences that are secondary consequences of the causative lesions of cancer. These are the abnormal patterns of normal protein expresson that define the malignant phenotype.

It has become increasingly evident that an enormous range of DNA mutations, that disrupt critical regulatory pathways that control cell growth, can cause cancer and reinforce the malignant state in cancerous cells. Although the DNA mutations are specific to the cancer cells, targeting the mutations or the

defective proteins that result from the DNA mutations may not be practical. It is likely that out of the estimated 140,000 genes in the human genome hundreds or perhaps thousands are capable of causing cancer. It is infeasible to prepare drugs that target each of these primary causes of cancer. In addition, many DNA mutations are known that induce malignant transformation by the loss of key regulatory proteins. In these cases the only way to distinguish the normal from malignant cells is by the secondary consequences that result from the absence of the regulatory protein. These consequences are the *abnormal patterns of normal protein expression* that define the malignant state. Although individually the proteins are normal and not unique to malignant cells the patterns of protein expression are highly specific to cancer. DNA mutations are the spark, but abnormal patterns of normal protein expression are the explosion and fire that is cancer. Anti-cancer drugs must be able to recognize the abnormal patterns of normal protein expression that define the malignant state. This is the purpose of the present invention.

These considerations highlight an important principle central to the problem of anti-cancer therapy. Anti-cancer therapies should be multifactorial unless directed against a causative lesion of cancer.

The hallmark of malignancy is uncontrolled cell proliferation and tissue invasion. The biochemical manifestation of these processes provides the basis for understanding and defining optimal tumor targeting. Neither the processes of cell replication nor the enzymology of tissue invasion (remodeling) are by themselves uniquely diagnostic of malignancy. But jointly, these processes likely

provide highly selective criteria to define effective targeting for the treatment of malignancy. The current class of multifunctional anti-cancer drugs provides the opportunity to have anti-cancer agents that are targeted simultaneously and jointly to both the proliferative and the invasive character of malignant cells.

In order to achieve tumor selectivity it is necessary to make drugs that can identify cancer cells. It is possible for a pathologist to distinguish malignant from normal cells in biopsies because the diagnostic criteria are multiple. Multiple factors such as cell size, shape, organization, location, and histochemistry allow differentiation between normal and malignant cells. In contrast, present anti-cancer drugs are essentially monofactorial directed against one property of malignancy such as cellular replication, invasiveness, or a tumor antigen. These individual properties are not unique to cancer cells and severely limit the selectivity of present anti-cancer drugs. The hallmark of malignancy is uncontrolled proliferation and invasiveness. The biochemistry of either alone is nonspecific. Jointly these properties characterize malignancy.

Although a single property or characteristic is not unique to malignant cells the pattern of expression of multiple such properties can provide almost absolute tumor specificity. Exquisite antitumor selectivity can be obtained by multifactorial drugs that target cells only if the cells <u>jointly</u> express multiple properties associated with the malignant phenotype. The present invention relates to technologies that can enable multifactorially targeted toxicity that is a consequence of multifactorial target recognition, effector action, or both. The present invention relates to a class of multifunctional, multifactorial drugs with

pattern recognition capabilities. The present technology also relates to compositions and methods by which selective multifactorial toxicity can be achieved by delivering multiple monofactorially targeted effector molecules. The invention also relates to key patterns of protein expression useful for selectively targeting cancer.

The present invention is a technology, which can allow the selective targeting of tumors with ultra-low doses of multiple drugs directed against multiple tumor targets. The high selectivity and high affinity of the drugs for tumor cells can enable the total dose of chemotherapy to be reduced thousands of times below current levels. The severe side effects currently associated with chemotherapy are not expected with ultra-low dose multiple drug therapy. Most importantly, the simultaneous use of multiple drugs directed against multiple tumor targets can potentially eliminate the problem of tumor resistance. The probability that a tumor could simultaneously develop resistance to ten independent drugs each capable of giving a 2 log reduction in tumor burden is essentially zero.

A second major application of the technology described in this patent is *targeted* immunotherapy in which an intense immune response directed against non-tumor antigens is specifically targeted to tumors to elicit tumor rejection. In addition, technology is described that can allow the targeted formation of neotumor antigens.

The present invention relates to the compositions, methods, and applications of a novel approach to selective cellular targeting. The purpose of this invention is

to enable the selective delivery and/or selective activation of effector molecules to target cells for diagnostic or therapeutic purposes. The present invention relates to multi-functional prodrugs or targeting vehicles wherein each functionality is capable of enhancing targeting selectivity, affinity, intracellular transport or activation. The present invention can be used to selectively target cells for diagnostic or therapeutic purposes. The principle applications are in the field of anti-cancer therapy. However, the applications are not limited to the delivery of antineoplastic drugs and can be employed in other applications where selective drug targeting is beneficial such as in the delivery of immunosuppressants.

Most current anti-cancer drugs are nonspecific or have low selectivity for tumor cells versus normal cells. The present invention seeks to address this problem by exploiting more than one property of tumor cells to define drug selectivity through the use of multi-functional delivery vehicles or prodrugs. Multifunctionality is also exploited to prevent the emergence of tumor drug resistance, and to selectively detoxify the drug in vital normal cells and to selectively toxify the drug in tumor cells.

Polymeric drugs and dendritic type drugs are well known, but do not provide an adequate solution to the seletive targeting and destruction of tumor cells even when connected to a targeting group such as a monoclonal antibody. The fundamental problem remains targeting specificity. The following references relates to this subject matter: WO 99/53951, 10/28/99, Martinez, et al., "Terminally-Branched Polymeric Linkers and Polymeric Conjugates Containing

the Same"; 5,783,178, 7/21/98 Kabanov, et al., "Polymer Linked Biological Agents."; Schacht E.H., et al., "Macromolecular Carriers for Drug Targeting," Wermuth C.G. (ed), *The Practice of Medicinal Chemistry,* Academic Press Limited, 1996, pp.717-736, the contents of which are incorporated herein by reference in their entirety.

The present invention also encompasses (embodiment ET1) A compound ET wherein E is comprised of one or more effector agents having pharmacological activity designated as "PA" and T is comprised of a targeting agent comprised of two or more groups each of which functions to specifically enhance the targeting selectivity by either increasing the pharmacological activity PA at targeted cells and/or decreasing the pharmacological activity PA at non-target cells;

and provided that at least one component of T is comprised of a group designated as a "selective targeting ligand" that binds specifically to a site designated as a "selective targeting receptor" on the target;

and wherein if a second selective targeting ligand is present in T then the first and second targeting ligands are able to bind simultaneously to two targeting receptor molecules;

and provided that T is not an antibody, or an analog or component of an antibody, or a complex of antibodies, or a bispecific antibody, or an analog of a bispecific antibody, or a natural protein, or a complex of natural proteins, or a protein, or a naturally occurring polymer, or a radiolabelled dimer, or a polymer

to which is attached at multiple sites one or more pharmacologically active compounds that mediate the same pharmacological activity PA.

The present invention also relates to the method of selectively targeting cells by the administration of said compound.

The present invention addresses the following critical aspects of antitumor drug function:

1.) Targeting specificity or the ability to localize the drug selectively to tumor cells.

2.) Transport of the targeted drug into the tumor cells.

3.) Triggering or activation to liberate the cytotoxic moiety at or in the tumor cell.

4.) Detoxification: the ability to selectively detoxify the drug to protect vital normal cells.

5.) Prevention of drug resistance.

Mechanism of Action

The mechanisms of actions and scientific basis of the present invention are described beginning on page 122.

The present invention encompasses a method (embodiment M1) to evoke a greater effector activity referred to as the pharmacological activity "PA"; at target cells compared to non-target cells;

wherein at the target cells there are present "m" different types of target molecules designated as (p1...pm); at least one of which is present at increased amounts compared to at a non-target cell, and wherein the type of the targeting molecule which is increased on the target cells compared to a non-target cell can be different for a different non-target cell;

and wherein at non-target cells there can be present the same types of target molecules (p1...pm);

wherein target molecules are biomolecules that are either target receptors or triggering agents;

wherein a target receptor is a chemical structure at the target cells that binds with a useful degree of specificity to a targeting ligand wherein said target receptor is present in increased amounts at the target cells compared to at some non-target cells;

and wherein a "triggering agent" is an enzyme, or biomolecule or other agent which is able to activate a trigger and which is increased at a target compared to at some non-target cells;

and wherein the method is comprised of contacting the cell or cell populations with one or more compounds designated as (C1...Cn), wherein at least one of the compounds has the structure E_1T_1; wherein E_1 is comprised of x effector

agents that evoke the pharmacological activity PA, and T_1 is comprised of the y different targeting ligands, and z different triggers, which increase the pharmacological activity PA at targeted cells and/or decrease the pharmacological activity PA at non-target cells;

and wherein a targeting ligand comprises a chemical structure, which binds with a degree of specificity to a targeting receptor that is enriched at a target cell compared to at a non-target cell;

and wherein a trigger is a chemical group which can undergo in vivo chemical modification either spontaneously or by a triggering agent with the modification leading to trigger activation that modulates the pharmacological activity of the drug;

and wherein the number m is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or about 20;

and wherein the number x is 1, 2, 3, 4, 5 or about 5;
and wherein the number y is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or about 10;
and wherein the number z is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or about 10;
and n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or about 10;

and wherein if n equals one then the sum of y and z is equal to or greater than m;

and wherein if n>1 the selectivity of the evoked response in targeted cells is not due solely to internalization and functional cooperation of the different effector groups inside the cells.

The present invention also encompasses (embodiment ET2) a multifunctional drug delivery vehicle which comprises a compound ET wherein E is comprised of one or more effector agents designated as E1...En wherein n =1,2,3,4, or 5 or about 5 and wherein these effector agents have pharmacological activity referred to as "PA"; and wherein T comprises a targeting agent which comprises: targeting ligands; or targeting ligands and triggers; and wherein T increases the pharmacological activity PA to a target cell compared to a non-target cell;

and wherein a targeting ligand is a group that binds selectively to a structure associated with the target referred to as a "targeting receptor";

and wherein a trigger is a group that upon in vivo modification by biomolecules referred to as "triggering agents" becomes activated and modulates the activity of ET;

and wherein at the target cells there are present "m" different types of target molecules designated as (p1...pm); at least one of which is present at increased amounts compared to at a non-target cell, and wherein the type of the targeting

molecule which is increased on the target cells compared to a non-target cell can be different for a different non-target cell;

and wherein at non-target cells there can be present the same types of target molecules (p1...pm);

wherein the number m is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

In a preferred embodiment the number m is 2, 3, 4, 5, or about 6.

The present invention also encompasses (embodiment ET3) a compound ET in which E is comprised of one or more effector agents having pharmacological activity designated as "PA" and wherein T comprises:

a) A group referred to as a "targeting ligand" which selectively binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell; and
b) One or more of the following:
   I. A targeting ligand which selectively binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell;
   II. A group, referred to as a "masked intracellular transport ligand" which can be modified in vivo to give a group referred to as an "intracellular transport ligand" which binds to a target cell receptor that actively transports bound ligands into the target cell;

III. A group referred to as a "trigger" that can be modified in vivo, wherein in vivo modification activates the trigger and modulates the pharmacological activity PA; and

IV. A group referred to as an "intracellular trapping ligand", which binds to one or more intracellular receptors or a group referred to as a "masked intracellular trapping ligand" which can be modified in vivo to give an "intracellular trapping ligand";

and wherein if a second targeting ligand is present in T then the first and second targeting ligands are able to bind simultaneously to two targeting receptor molecules;

and wherein if T is comprised solely of a targeting ligand a trigger and in vivo modification of the trigger increases the pharmacological activity PA then the in vivo modification which activates the trigger is caused by an enzyme or enzymatic activity that is increased at target cells or decreased at non-target cells;

and wherein if T is comprised solely of a targeting ligand a trigger and in vivo modification of the trigger decreases the pharmacological activity PA then the in vivo modification which activates the trigger is caused by an enzyme or enzymatic activity that is decreased at target cells or increased at non-target cells;

and provided that T is not an antibody, or an analog or component of an antibody, or a complex of antibodies, or a bispecific antibody, or an analog of a bispecific antibody, or a natural protein, or a complex of natural proteins, or a protein, or a naturally occurring polymer, or a radiolabelled dimer, or a polymer to which is attached at multiple sites one or more pharmacologically active compounds that evoke the same pharmacological activity PA.

A preferred embodiment, of all the prior embodiments of ET, comprises ET wherein ET evokes a greater pharmacological activity PA at the target cell compared to a non-target cell and wherein this target cell selectivity is due to functional cooperation between the components of ET and not due to any single component of ET acting alone.

A preferred embodiment comprises ET wherein ET is comprised of a compound in which the targeting ligand selectively binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell wherein the concentration of the target receptor is greater on the surface of the target cell or in the microenvironment of the target cell than on the surface or in the microenvironment of non-target cells.

A preferred embodiment of the present invention (embodiment ET4) comprises ET wherein ET is comprised of a compound with two or more targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell wherein the target has an increased amount of that target receptor compared to a non-target

cell that binds to a second targeting ligand of the compound. Generally, the increased amount is greater than about two times or greater than about 5 times, or greater than about 10 times. A preferred embodiment is comprised of ET in which at least one of the targeting ligands binds to a receptor that is absent or essentially absent from a non-targeted cell.

Methods for detecting increased amounts of receptors are well known to one skilled in the arts and include immunohistochemistry, radioimmunoassays, enzymatic assays, and a variety of nucleic acid hybridization techniques.

A preferred embodiment (embodiment ET5) comprises ET wherein ET is comprised of a compound with two or more targeting ligands that binds to a target cell with an affinity that is greater than a non-target cell presenting a target receptor(s) that bind to the targeting ligands of said compound. In preferred embodiments the above mentioned binding affinity to the target cell is at least about 2-5 times greater, or at least about 5-10 times greater, or at least about10-50 times greater, or at least about 50-500 times greater, or at least about 500-5000 times greater, or at least about 5000-50,000 times greater, or at least about 50,000-1,000,000 times greater or more then 1 million times greater than to the non-target cell.

A preferred embodiment (embodiment ET6) comprises ET wherein ET is comprised of a drug with binding affinity to target cells that is approximately the same as to a population of non-target cells however said population of non-target cells have decreased sensitivity to the effects of the effector agent

because said normal cells have decreased levels of an intracellular trapping receptor, or decreased sensitivity to the effector agent, or decreased levels of a specific protein necessary for neoantigen formation, or decreased levels of an enzyme that activates a trigger that increases the toxicity of ET, or increased levels of an enzyme that activates a trigger that decreases the toxicity of ET, or by virtue of said normal cells being located in the body at a site such as the brain where the drug ET cannot penetrate to a significant degree.

A preferred embodiment of ET is comprised of a compound in which the intracellular trapping ligand selectively binds to one or more intracellular receptors wherein the concentration of the intracellular receptors is greater in target cells than in non-target cells.

A preferred embodiment of ET is comprised of a compound with a trigger that increases the pharmacological activity PA upon in vivo modification and wherein the in vivo modification that activates the trigger is caused by an enzyme or enzymatic activity that is increased at target cells or decreased at non-target cells.

A preferred embodiment of ET is comprised of a compound with a trigger that decreases the pharmacological activity PA upon in vivo modification and wherein the in vivo modification that activates the trigger is caused by an enzyme or enzymatic activity that is decreased at target cells or increased at non-target cells.

A preferred embodiment of ET is comprised of a compound in which the intracellular transport ligand binds to a molecule referred to as a "transporter molecule" to form a complex and wherein this complex binds to a target cell receptor that actively transports bound ligands into the target cell.

A preferred embodiment of ET is comprised of a compound in which the concentration of transporter molecules is increased at the surface of target cells compared to non-target cells.

A preferred embodiment of ET is comprised of a compound with two targeting ligands that selectively bind to target receptors on the surface of the target cell or in the microenvironment of the target cell wherein the concentration of the target receptors is greater on the surface of the target cell or in the microenvironment of the target cell than on the surface or in the microenvironment of non-target cells. In a preferred embodiment these targeting ligands are the same. In another preferred embodiment these targeting ligands are different and bind to different types of targeting receptors.

A preferred embodiment of ET is comprised of a compound with three targeting ligands that selectively bind to target receptors on the surface of the target cell or in the microenvironment of the target cell wherein the concentration of the target receptors is greater on the surface of the target cell or in the microenvironment of the target cell than on the surface or in the microenvironment of non-target cells. In a preferred embodiment these targeting

ligands are the same. In another preferred embodiment these targeting ligands are different and bind to different types of targeting receptors.

A preferred embodiment of ET is comprised of a compound with four targeting ligands that selectively bind to target receptors on the surface of the target cell or in the microenvironment of the target cell wherein the concentration of the target receptors is greater on the surface of the target cell or in the microenvironment of the target cell than on the surface or in the microenvironment of non-target cells. In a preferred embodiment these targeting ligands are the same. In another preferred embodiment these targeting ligands are different and bind to different types of targeting receptors.

Another preferred embodiment of ET is comprised of a compound with two or more targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell wherein the target has an increased amount of that target receptor compared to a non-target cell that binds to a second targeting ligand of the compound. A preferred embodiment of this embodiment comprises a compound with two different targeting ligands that bind to two different targeting receptors. Another preferred embodiment of this embodiment comprises a compound with three different targeting ligands that bind to three different targeting receptors. Another preferred embodiment of this embodiment comprises a compound with four different targeting ligands that bind to four different targeting receptors.

A preferred embodiment (embodiment ET7) of ET is comprised of the following groups:

I. N1 targeting ligands, which can differ;

II. N2 masked intracellular transport ligands which can differ;

III. N3 triggers, which can differ, designated "detoxification triggers" wherein activation of the trigger decreases the pharmacological activity PA;

IV. N4 effector agents which can differ;

V. N5 triggers which can differ, wherein activation of the trigger increases the pharmacological activity PA;

VI. N6 intracellular trapping ligands or masked intracellular trapping ligands, which can differ;

and wherein:

N1 = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or about 10; N2 = 0, 1, 2, 3, 4, or about 4;

N3 = 0, 1, 2, 3, 4, 5, or about 5; N4 = 1, 2, 3, 4, 5, or about 5;

N5 = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or about 10; and

N6 = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or about 10;

and wherein the components are covalently coupled directly or by one or more linkers, and wherein the connectivity between groups can vary provided that the functionality of the different components remains intact and wherein the function of ligands is to bind to their respective receptors; the function of the triggers is to be activated and modulate drug activity, and the function of the effector agent is to evoke the pharmacological activity PA;

and wherein the linker lengths can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, ...300 bond lengths or about 300 bond lengths; wherein the (...) are meant to represent the continuation of the sequence of numbers up to 300.

The connectivity is not critical because the target molecules that the groups interact with are not rigidly fixed in space.

Detailed descriptions of each of the components of ET are given in later sections

A preferred embodiment (embodiment ET8) comprises ET with

N1 =1, 2, 3, or 4;	N2 =0, 1, or 2;
N3 =0, 1, or 2;	N4 = 1, 2, or 3;
N5 =0, 1, 2, or 3;	N6 =1, 2, or 3;

Additional preferred embodiments of ET (embodiment ET8.X wherein X=# in the list below) are listed on each line below wherein:

1) N1=1, N2=0, N3=1, N4=1, N5=0, and N6=0

2) N1=1, N2=0, N3=0, N4=2, N5=0, and N6=0

3) N1=1, N2=0, N3=0, N4=3, N5=0, and N6=0

4) N1=1, N2=0, N3=0, N4=1, N5=1, and N6=0

5) N1=1, N2=0, N3=0, N4=1, N5=2, and N6=0

6) N1=1, N2=0, N3=0, N4=1, N5=3, and N6=0

7) N1=1, N2=0, N3=0, N4=1, N5=0, and N6=1

8) N1=1, N2=0, N3=1, N4=2, N5=0, and N6=0

9) N1=1, N2=0, N3=1, N4=3, N5=0, and N6=0

10) N1=1, N2=0, N3=1, N4=1, N5=1, and N6=0

11) N1=1, N2=0, N3=1, N4=1, N5=2, and N6=0

12) N1=1, N2=0, N3=1, N4=1, N5=3, and N6=0

13) N1=1, N2=0, N3=1, N4=1, N5=0, and N6=1

14) N1=1, N2=0, N3=1, N4=2, N5=1, and N6=0

15) N1=1, N2=0, N3=1, N4=2, N5=1, and N6=1

16) N1=1, N2=0, N3=1, N4=2, N5=2, and N6=0

17) N1=1, N2=0, N3=1, N4=2, N5=2, and N6=1

18) N1=1, N2=0, N3=1, N4=2, N5=3, and N6=0

19) N1=1, N2=0, N3=1, N4=2, N5=3, and N6=1

20) N1=1, N2=0, N3=1, N4=2, N5=0, and N6=1

21) N1=1, N2=0, N3=1, N4=3, N5=1, and N6=0

22) N1=1, N2=0, N3=1, N4=3, N5=1, and N6=1

23) N1=1, N2=0, N3=1, N4=3, N5=2, and N6=0

24) N1=1, N2=0, N3=1, N4=3, N5=2, and N6=1

25) N1=1, N2=0, N3=1, N4=3, N5=3, and N6=0

26) N1=1, N2=0, N3=1, N4=3, N5=3, and N6=1

27) N1=1, N2=0, N3=1, N4=3, N5=0, and N6=1

28) N1=1, N2=0, N3=1, N4=1, N5=1, and N6=1

29) N1=1, N2=0, N3=1, N4=1, N5=2, and N6=1

30) N1=1, N2=0, N3=1, N4=1, N5=3, and N6=1

31) N1=1, N2=1, N3=0, N4=1, N5=0, and N6=0

32) N1=1, N2=1, N3=0, N4=2, N5=0, and N6=0

33) N1=1, N2=1, N3=0, N4=3, N5=0, and N6=0

34) N1=1, N2=1, N3=0, N4=1, N5=1, and N6=0

35) N1=1, N2=1, N3=0, N4=1, N5=2, and N6=0

36) N1=1, N2=1, N3=0, N4=1, N5=3, and N6=0

37) N1=1, N2=1, N3=0, N4=1, N5=0, and N6=1

38) N1=1, N2=1, N3=0, N4=2, N5=1, and N6=0

39) N1=1, N2=1, N3=0, N4=2, N5=1, and N6=1

40) N1=1, N2=1, N3=0, N4=2, N5=2, and N6=0

41) N1=1, N2=1, N3=0, N4=2, N5=2, and N6=1

42) N1=1, N2=1, N3=0, N4=2, N5=3, and N6=0

43) N1=1, N2=1, N3=0, N4=2, N5=3, and N6=1

44) N1=1, N2=1, N3=0, N4=2, N5=0, and N6=1

45) N1=1, N2=1, N3=0, N4=3, N5=1, and N6=0

46) N1=1, N2=1, N3=0, N4=3, N5=1, and N6=1

47) N1=1, N2=1, N3=0, N4=3, N5=2, and N6=0

48) N1=1, N2=1, N3=0, N4=3, N5=2, and N6=1

49) N1=1, N2=1, N3=0, N4=3, N5=3, and N6=0

50) N1=1, N2=1, N3=0, N4=3, N5=3, and N6=1

51) N1=1, N2=1, N3=0, N4=3, N5=0, and N6=1

52) N1=1, N2=1, N3=0, N4=1, N5=1, and N6=1

53) N1=1, N2=1, N3=0, N4=1, N5=2, and N6=1

54) N1=1, N2=1, N3=0, N4=1, N5=3, and N6=1

55) N1=1, N2=1, N3=1, N4=1, N5=0, and N6=0

56) N1=1, N2=1, N3=1, N4=2, N5=0, and N6=0

57) N1=1, N2=1, N3=1, N4=3, N5=0, and N6=0

58) N1=1, N2=1, N3=1, N4=1, N5=1, and N6=0

59) N1=1, N2=1, N3=1, N4=1, N5=2, and N6=0

60) N1=1, N2=1, N3=1, N4=1, N5=3, and N6=0

61) N1=1, N2=1, N3=1, N4=1, N5=0, and N6=1

62) N1=1, N2=1, N3=1, N4=2, N5=1, and N6=0

63) N1=1, N2=1, N3=1, N4=2, N5=1, and N6=1

64) N1=1, N2=1, N3=1, N4=2, N5=2, and N6=0

65) N1=1, N2=1, N3=1, N4=2, N5=2, and N6=1

66) N1=1, N2=1, N3=1, N4=2, N5=3, and N6=0

67) N1=1, N2=1, N3=1, N4=2, N5=3, and N6=1

68) N1=1, N2=1, N3=1, N4=2, N5=0, and N6=1

69) N1=1, N2=1, N3=1, N4=3, N5=1, and N6=0

70) N1=1, N2=1, N3=1, N4=3, N5=1, and N6=1

71) N1=1, N2=1, N3=1, N4=3, N5=2, and N6=0

72) N1=1, N2=1, N3=1, N4=3, N5=2, and N6=1

73) N1=1, N2=1, N3=1, N4=3, N5=3, and N6=0

74) N1=1, N2=1, N3=1, N4=3, N5=3, and N6=1

75) N1=1, N2=1, N3=1, N4=3, N5=0, and N6=1

76) N1=1, N2=1, N3=1, N4=1, N5=1, and N6=1

77) N1=1, N2=1, N3=1, N4=1, N5=2, and N6=1

78) N1=1, N2=1, N3=1, N4=1, N5=3, and N6=1

79) N1=1, N2=0, N3=0, N4=2, N5=1, and N6=0

80) N1=1, N2=0, N3=0, N4=2, N5=2, and N6=0

81) N1=1, N2=0, N3=0, N4=2, N5=3, and N6=0

82) N1=1, N2=0, N3=0, N4=2, N5=0, and N6=1

83) N1=1, N2=0, N3=0, N4=3, N5=1, and N6=0

84) N1=1, N2=0, N3=0, N4=3, N5=2, and N6=0

85) N1=1, N2=0, N3=0, N4=3, N5=3, and N6=0

86) N1=1, N2=0, N3=0, N4=3, N5=0, and N6=1

87) N1=1, N2=0, N3=0, N4=1, N5=2, and N6=1

88) N1=1, N2=0, N3=0, N4=1, N5=3, and N6=1

89) N1=1, N2=0, N3=0, N4=2, N5=1, and N6=1

90) N1=1, N2=0, N3=0, N4=2, N5=2, and N6=1

91) N1=1, N2=0, N3=0, N4=2, N5=3, and N6=1

92) N1=1, N2=0, N3=0, N4=3, N5=1, and N6=1

93) N1=1, N2=0, N3=0, N4=3, N5=2, and N6=1

94) N1=1, N2=0, N3=0, N4=3, N5=3, and N6=1

95) N1=1, N2=0, N3=0, N4=1, N5=1, and N6=1

96) N1=2, N2=0, N3=0, N4=1, N5=0, and N6=0

97) N1=2, N2=0, N3=1, N4=1, N5=0, and N6=0

98) N1=2, N2=0, N3=0, N4=2, N5=0, and N6=0

99) N1=2, N2=0, N3=0, N4=3, N5=0, and N6=0

100) N1=2, N2=0, N3=0, N4=1, N5=1, and N6=0

101) N1=2, N2=0, N3=0, N4=1, N5=2, and N6=0

102) N1=2, N2=0, N3=0, N4=1, N5=3, and N6=0

103) N1=2, N2=0, N3=0, N4=1, N5=0, and N6=1

104) N1=2, N2=0, N3=1, N4=2, N5=0, and N6=0

105) N1=2, N2=0, N3=1, N4=3, N5=0, and N6=0

106) N1=2, N2=0, N3=1, N4=1, N5=1, and N6=0

107) N1=2, N2=0, N3=1, N4=1, N5=2, and N6=0

108) N1=2, N2=0, N3=1, N4=1, N5=3, and N6=0

109) N1=2, N2=0, N3=1, N4=1, N5=0, and N6=1

110) N1=2, N2=0, N3=1, N4=2, N5=1, and N6=0

111) N1=2, N2=0, N3=1, N4=2, N5=1, and N6=1

112) N1=2, N2=0, N3=1, N4=2, N5=2, and N6=0

113) N1=2, N2=0, N3=1, N4=2, N5=2, and N6=1

114) N1=2, N2=0, N3=1, N4=2, N5=3, and N6=0

115) N1=2, N2=0, N3=1, N4=2, N5=3, and N6=1

116) N1=2, N2=0, N3=1, N4=2, N5=0, and N6=1

117) N1=2, N2=0, N3=1, N4=3, N5=1, and N6=0

118) N1=2, N2=0, N3=1, N4=3, N5=1, and N6=1

119) N1=2, N2=0, N3=1, N4=3, N5=2, and N6=0

120) N1=2, N2=0, N3=1, N4=3, N5=2, and N6=1

121) N1=2, N2=0, N3=1, N4=3, N5=3, and N6=0

122) N1=2, N2=0, N3=1, N4=3, N5=3, and N6=1

123) N1=2, N2=0, N3=1, N4=3, N5=0, and N6=1

124) N1=2, N2=0, N3=1, N4=1, N5=1, and N6=1

125) N1=2, N2=0, N3=1, N4=1, N5=2, and N6=1

126) N1=2, N2=0, N3=1, N4=1, N5=3, and N6=1

127) N1=2, N2=1, N3=0, N4=1, N5=0, and N6=0

128) N1=2, N2=1, N3=0, N4=2, N5=0, and N6=0

129) N1=2, N2=1, N3=0, N4=3, N5=0, and N6=0

130) N1=2, N2=1, N3=0, N4=1, N5=1, and N6=0

131) N1=2, N2=1, N3=0, N4=1, N5=2, and N6=0

132) N1=2, N2=1, N3=0, N4=1, N5=3, and N6=0

133) N1=2, N2=1, N3=0, N4=1, N5=0, and N6=1

134) N1=2, N2=1, N3=0, N4=2, N5=1, and N6=0

135) N1=2, N2=1, N3=0, N4=2, N5=1, and N6=1

136) N1=2, N2=1, N3=0, N4=2, N5=2, and N6=0

137) N1=2, N2=1, N3=0, N4=2, N5=2, and N6=1

138) N1=2, N2=1, N3=0, N4=2, N5=3, and N6=0

139) N1=2, N2=1, N3=0, N4=2, N5=3, and N6=1

140) N1=2, N2=1, N3=0, N4=2, N5=0, and N6=1

141) N1=2, N2=1, N3=0, N4=3, N5=1, and N6=0

142) N1=2, N2=1, N3=0, N4=3, N5=1, and N6=1

143) N1=2, N2=1, N3=0, N4=3, N5=2, and N6=0

144) N1=2, N2=1, N3=0, N4=3, N5=2, and N6=1

145) N1=2, N2=1, N3=0, N4=3, N5=3, and N6=0

146) N1=2, N2=1, N3=0, N4=3, N5=3, and N6=1

147) N1=2, N2=1, N3=0, N4=3, N5=0, and N6=1

148) N1=2, N2=1, N3=0, N4=1, N5=1, and N6=1

149) N1=2, N2=1, N3=0, N4=1, N5=2, and N6=1

150) N1=2, N2=1, N3=0, N4=1, N5=3, and N6=1

151) N1=2, N2=1, N3=1, N4=1, N5=0, and N6=0

152) N1=2, N2=1, N3=1, N4=2, N5=0, and N6=0

153) N1=2, N2=1, N3=1, N4=3, N5=0, and N6=0

154) N1=2, N2=1, N3=1, N4=1, N5=1, and N6=0

155) N1=2, N2=1, N3=1, N4=1, N5=2, and N6=0

156) N1=2, N2=1, N3=1, N4=1, N5=3, and N6=0

157) N1=2, N2=1, N3=1, N4=1, N5=0, and N6=1

158) N1=2, N2=1, N3=1, N4=2, N5=1, and N6=0

159) N1=2, N2=1, N3=1, N4=2, N5=1, and N6=1

160) N1=2, N2=1, N3=1, N4=2, N5=2, and N6=0

161) N1=2, N2=1, N3=1, N4=2, N5=2, and N6=1

162) N1=2, N2=1, N3=1, N4=2, N5=3, and N6=0

163) N1=2, N2=1, N3=1, N4=2, N5=3, and N6=1

164) N1=2, N2=1, N3=1, N4=2, N5=0, and N6=1

165) N1=2, N2=1, N3=1, N4=3, N5=1, and N6=0

166) N1=2, N2=1, N3=1, N4=3, N5=1, and N6=1

167) N1=2, N2=1, N3=1, N4=3, N5=2, and N6=0

168) N1=2, N2=1, N3=1, N4=3, N5=2, and N6=1

169) N1=2, N2=1, N3=1, N4=3, N5=3, and N6=0

170) N1=2, N2=1, N3=1, N4=3, N5=3, and N6=1

171) N1=2, N2=1, N3=1, N4=3, N5=0, and N6=1

172) N1=2, N2=1, N3=1, N4=1, N5=1, and N6=1

173) N1=2, N2=1, N3=1, N4=1, N5=2, and N6=1

174) N1=2, N2=1, N3=1, N4=1, N5=3, and N6=1

175) N1=2, N2=0, N3=0, N4=2, N5=1, and N6=0

176) N1=2, N2=0, N3=0, N4=2, N5=2, and N6=0

177) N1=2, N2=0, N3=0, N4=2, N5=3, and N6=0

178) N1=2, N2=0, N3=0, N4=2, N5=0, and N6=1

179) N1=2, N2=0, N3=0, N4=3, N5=1, and N6=0

180) N1=2, N2=0, N3=0, N4=3, N5=2, and N6=0

181) N1=2, N2=0, N3=0, N4=3, N5=3, and N6=0

182) N1=2, N2=0, N3=0, N4=3, N5=0, and N6=1

183) N1=2, N2=0, N3=0, N4=1, N5=2, and N6=1

184) N1=2, N2=0, N3=0, N4=1, N5=3, and N6=1

185) N1=2, N2=0, N3=0, N4=2, N5=1, and N6=1

186) N1=2, N2=0, N3=0, N4=2, N5=2, and N6=1

187) N1=2, N2=0, N3=0, N4=2, N5=3, and N6=1

188) N1=2, N2=0, N3=0, N4=3, N5=1, and N6=1

189) N1=2, N2=0, N3=0, N4=3, N5=2, and N6=1

190) N1=2, N2=0, N3=0, N4=3, N5=3, and N6=1

191) N1=2, N2=0, N3=0, N4=1, N5=1, and N6=1

192) N1=3, N2=0, N3=0, N4=1, N5=0, and N6=0

193) N1=3, N2=0, N3=1, N4=1, N5=0, and N6=0

194) N1=3, N2=0, N3=0, N4=2, N5=0, and N6=0

195) N1=3, N2=0, N3=0, N4=3, N5=0, and N6=0

196) N1=3, N2=0, N3=0, N4=1, N5=1, and N6=0

197) N1=3, N2=0, N3=0, N4=1, N5=2, and N6=0

198) N1=3, N2=0, N3=0, N4=1, N5=3, and N6=0

199) N1=3, N2=0, N3=0, N4=1, N5=0, and N6=1

200) N1=3, N2=0, N3=1, N4=2, N5=0, and N6=0

201) N1=3, N2=0, N3=1, N4=3, N5=0, and N6=0

202) N1=3, N2=0, N3=1, N4=1, N5=1, and N6=0

203) N1=3, N2=0, N3=1, N4=1, N5=2, and N6=0

204) N1=3, N2=0, N3=1, N4=1, N5=3, and N6=0

205) N1=3, N2=0, N3=1, N4=1, N5=0, and N6=1

206) N1=3, N2=0, N3=1, N4=2, N5=1, and N6=0

207) N1=3, N2=0, N3=1, N4=2, N5=1, and N6=1

208) N1=3, N2=0, N3=1, N4=2, N5=2, and N6=0

209) N1=3, N2=0, N3=1, N4=2, N5=2, and N6=1

210) N1=3, N2=0, N3=1, N4=2, N5=3, and N6=0

211) N1=3, N2=0, N3=1, N4=2, N5=3, and N6=1

212) N1=3, N2=0, N3=1, N4=2, N5=0, and N6=1

213) N1=3, N2=0, N3=1, N4=3, N5=1, and N6=0

214) N1=3, N2=0, N3=1, N4=3, N5=1, and N6=1

215) N1=3, N2=0, N3=1, N4=3, N5=2, and N6=0

216) N1=3, N2=0, N3=1, N4=3, N5=2, and N6=1

217) N1=3, N2=0, N3=1, N4=3, N5=3, and N6=0

218) N1=3, N2=0, N3=1, N4=3, N5=3, and N6=1

219) N1=3, N2=0, N3=1, N4=3, N5=0, and N6=1

220) N1=3, N2=0, N3=1, N4=1, N5=1, and N6=1

221) N1=3, N2=0, N3=1, N4=1, N5=2, and N6=1

222) N1=3, N2=0, N3=1, N4=1, N5=3, and N6=1

223) N1=3, N2=1, N3=0, N4=1, N5=0, and N6=0

224) N1=3, N2=1, N3=0, N4=2, N5=0, and N6=0

225) N1=3, N2=1, N3=0, N4=3, N5=0, and N6=0

226) N1=3, N2=1, N3=0, N4=1, N5=1, and N6=0

227) N1=3, N2=1, N3=0, N4=1, N5=2, and N6=0

228) N1=3, N2=1, N3=0, N4=1, N5=3, and N6=0

229) N1=3, N2=1, N3=0, N4=1, N5=0, and N6=1

230) N1=3, N2=1, N3=0, N4=2, N5=1, and N6=0

231) N1=3, N2=1, N3=0, N4=2, N5=1, and N6=1

232) N1=3, N2=1, N3=0, N4=2, N5=2, and N6=0

233) N1=3, N2=1, N3=0, N4=2, N5=2, and N6=1

234) N1=3, N2=1, N3=0, N4=2, N5=3, and N6=0

235) N1=3, N2=1, N3=0, N4=2, N5=3, and N6=1

236) N1=3, N2=1, N3=0, N4=2, N5=0, and N6=1

237) N1=3, N2=1, N3=0, N4=3, N5=1, and N6=0

238) N1=3, N2=1, N3=0, N4=3, N5=1, and N6=1

239) N1=3, N2=1, N3=0, N4=3, N5=2, and N6=0

240) N1=3, N2=1, N3=0, N4=3, N5=2, and N6=1

241) N1=3, N2=1, N3=0, N4=3, N5=3, and N6=0

242) N1=3, N2=1, N3=0, N4=3, N5=3, and N6=1

243) N1=3, N2=1, N3=0, N4=3, N5=0, and N6=1

244) N1=3, N2=1, N3=0, N4=1, N5=1, and N6=1

245) N1=3, N2=1, N3=0, N4=1, N5=2, and N6=1

246) N1=3, N2=1, N3=0, N4=1, N5=3, and N6=1

247) N1=3, N2=1, N3=1, N4=1, N5=0, and N6=0

248) N1=3, N2=1, N3=1, N4=2, N5=0, and N6=0

249) N1=3, N2=1, N3=1, N4=3, N5=0, and N6=0

250) N1=3, N2=1, N3=1, N4=1, N5=1, and N6=0

251) N1=3, N2=1, N3=1, N4=1, N5=2, and N6=0

252) N1=3, N2=1, N3=1, N4=1, N5=3, and N6=0

253) N1=3, N2=1, N3=1, N4=1, N5=0, and N6=1

254) N1=3, N2=1, N3=1, N4=2, N5=1, and N6=0

255) N1=3, N2=1, N3=1, N4=2, N5=1, and N6=1

256) N1=3, N2=1, N3=1, N4=2, N5=2, and N6=0

257) N1=3, N2=1, N3=1, N4=2, N5=2, and N6=1

258) N1=3, N2=1, N3=1, N4=2, N5=3, and N6=0

259) N1=3, N2=1, N3=1, N4=2, N5=3, and N6=1

260) N1=3, N2=1, N3=1, N4=2, N5=0, and N6=1

261) N1=3, N2=1, N3=1, N4=3, N5=1, and N6=0

262) N1=3, N2=1, N3=1, N4=3, N5=1, and N6=1

263) N1=3, N2=1, N3=1, N4=3, N5=2, and N6=0

264) N1=3, N2=1, N3=1, N4=3, N5=2, and N6=1

265) N1=3, N2=1, N3=1, N4=3, N5=3, and N6=0

266) N1=3, N2=1, N3=1, N4=3, N5=3, and N6=1

267) N1=3, N2=1, N3=1, N4=3, N5=0, and N6=1

268) N1=3, N2=1, N3=1, N4=1, N5=1, and N6=1

269) N1=3, N2=1, N3=1, N4=1, N5=2, and N6=1

270) N1=3, N2=1, N3=1, N4=1, N5=3, and N6=1

271) N1=3, N2=0, N3=0, N4=2, N5=1, and N6=0

272) N1=3, N2=0, N3=0, N4=2, N5=2, and N6=0

273) N1=3, N2=0, N3=0, N4=2, N5=3, and N6=0

274) N1=3, N2=0, N3=0, N4=2, N5=0, and N6=1

275) N1=3, N2=0, N3=0, N4=3, N5=1, and N6=0

276) N1=3, N2=0, N3=0, N4=3, N5=2, and N6=0

277) N1=3, N2=0, N3=0, N4=3, N5=3, and N6=0

278) N1=3, N2=0, N3=0, N4=3, N5=0, and N6=1

279) N1=3, N2=0, N3=0, N4=1, N5=2, and N6=1

280) N1=3, N2=0, N3=0, N4=1, N5=3, and N6=1

281) N1=3, N2=0, N3=0, N4=2, N5=1, and N6=1

282) N1=3, N2=0, N3=0, N4=2, N5=2, and N6=1

283) N1=3, N2=0, N3=0, N4=2, N5=3, and N6=1

284) N1=3, N2=0, N3=0, N4=3, N5=1, and N6=1

285) N1=3, N2=0, N3=0, N4=3, N5=2, and N6=1

286) N1=3, N2=0, N3=0, N4=3, N5=3, and N6=1

287) N1=3, N2=0, N3=0, N4=1, N5=1, and N6=1

288) N1=4, N2=0, N3=0, N4=1, N5=0, and N6=0

289) N1=4, N2=0, N3=1, N4=1, N5=0, and N6=0

290) N1=4, N2=0, N3=0, N4=2, N5=0, and N6=0

291) N1=4, N2=0, N3=0, N4=3, N5=0, and N6=0

292) N1=4, N2=0, N3=0, N4=1, N5=1, and N6=0

293) N1=4, N2=0, N3=0, N4=1, N5=2, and N6=0

294) N1=4, N2=0, N3=0, N4=1, N5=3, and N6=0

295) N1=4, N2=0, N3=0, N4=1, N5=0, and N6=1

296) N1=4, N2=0, N3=1, N4=2, N5=0, and N6=0

297) N1=4, N2=0, N3=1, N4=3, N5=0, and N6=0

298) N1=4, N2=0, N3=1, N4=1, N5=1, and N6=0

299) N1=4, N2=0, N3=1, N4=1, N5=2, and N6=0

300) N1=4, N2=0, N3=1, N4=1, N5=3, and N6=0

301) N1=4, N2=0, N3=1, N4=1, N5=0, and N6=1

302) N1=4, N2=0, N3=1, N4=2, N5=1, and N6=0

303) N1=4, N2=0, N3=1, N4=2, N5=1, and N6=1

304) N1=4, N2=0, N3=1, N4=2, N5=2, and N6=0

305) N1=4, N2=0, N3=1, N4=2, N5=2, and N6=1

306) N1=4, N2=0, N3=1, N4=2, N5=3, and N6=0

307) N1=4, N2=0, N3=1, N4=2, N5=3, and N6=1

308) N1=4, N2=0, N3=1, N4=2, N5=0, and N6=1

309) N1=4, N2=0, N3=1, N4=3, N5=1, and N6=0

310) N1=4, N2=0, N3=1, N4=3, N5=1, and N6=1

311) N1=4, N2=0, N3=1, N4=3, N5=2, and N6=0

312) N1=4, N2=0, N3=1, N4=3, N5=2, and N6=1

313) N1=4, N2=0, N3=1, N4=3, N5=3, and N6=0

314) N1=4, N2=0, N3=1, N4=3, N5=3, and N6=1

315) N1=4, N2=0, N3=1, N4=3, N5=0, and N6=1

316) N1=4, N2=0, N3=1, N4=1, N5=1, and N6=1

317) N1=4, N2=0, N3=1, N4=1, N5=2, and N6=1

318) N1=4, N2=0, N3=1, N4=1, N5=3, and N6=1

319) N1=4, N2=1, N3=0, N4=1, N5=0, and N6=0

320) N1=4, N2=1, N3=0, N4=2, N5=0, and N6=0

321) N1=4, N2=1, N3=0, N4=3, N5=0, and N6=0

322) N1=4, N2=1, N3=0, N4=1, N5=1, and N6=0

323) N1=4, N2=1, N3=0, N4=1, N5=2, and N6=0

324) N1=4, N2=1, N3=0, N4=1, N5=3, and N6=0

325) N1=4, N2=1, N3=0, N4=1, N5=0, and N6=1

326) N1=4, N2=1, N3=0, N4=2, N5=1, and N6=0

327) N1=4, N2=1, N3=0, N4=2, N5=1, and N6=1

328) N1=4, N2=1, N3=0, N4=2, N5=2, and N6=0

329) N1=4, N2=1, N3=0, N4=2, N5=2, and N6=1

330) N1=4, N2=1, N3=0, N4=2, N5=3, and N6=0

331) N1=4, N2=1, N3=0, N4=2, N5=3, and N6=1

332) N1=4, N2=1, N3=0, N4=2, N5=0, and N6=1

333) N1=4, N2=1, N3=0, N4=3, N5=1, and N6=0

334) N1=4, N2=1, N3=0, N4=3, N5=1, and N6=1

335) N1=4, N2=1, N3=0, N4=3, N5=2, and N6=0

336) N1=4, N2=1, N3=0, N4=3, N5=2, and N6=1

337) N1=4, N2=1, N3=0, N4=3, N5=3, and N6=0

338) N1=4, N2=1, N3=0, N4=3, N5=3, and N6=1

339) N1=4, N2=1, N3=0, N4=3, N5=0, and N6=1

340) N1=4, N2=1, N3=0, N4=1, N5=1, and N6=1

341) N1=4, N2=1, N3=0, N4=1, N5=2, and N6=1

342) N1=4, N2=1, N3=0, N4=1, N5=3, and N6=1

343) N1=4, N2=1, N3=1, N4=1, N5=0, and N6=0

344) N1=4, N2=1, N3=1, N4=2, N5=0, and N6=0

345) N1=4, N2=1, N3=1, N4=3, N5=0, and N6=0

346) N1=4, N2=1, N3=1, N4=1, N5=1, and N6=0

347) N1=4, N2=1, N3=1, N4=1, N5=2, and N6=0

348) N1=4, N2=1, N3=1, N4=1, N5=3, and N6=0

349) N1=4, N2=1, N3=1, N4=1, N5=0, and N6=1

350) N1=4, N2=1, N3=1, N4=2, N5=1, and N6=0

351) N1=4, N2=1, N3=1, N4=2, N5=1, and N6=1

352) N1=4, N2=1, N3=1, N4=2, N5=2, and N6=0

353) N1=4, N2=1, N3=1, N4=2, N5=2, and N6=1

354) N1=4, N2=1, N3=1, N4=2, N5=3, and N6=0

355) N1=4, N2=1, N3=1, N4=2, N5=3, and N6=1

356) N1=4, N2=1, N3=1, N4=2, N5=0, and N6=1

357) N1=4, N2=1, N3=1, N4=3, N5=1, and N6=0

358) N1=4, N2=1, N3=1, N4=3, N5=1, and N6=1

359) N1=4, N2=1, N3=1, N4=3, N5=2, and N6=0

360) N1=4, N2=1, N3=1, N4=3, N5=2, and N6=1

361) N1=4, N2=1, N3=1, N4=3, N5=3, and N6=0

362) N1=4, N2=1, N3=1, N4=3, N5=3, and N6=1

363) N1=4, N2=1, N3=1, N4=3, N5=0, and N6=1

364) N1=4, N2=1, N3=1, N4=1, N5=1, and N6=1

365) N1=4, N2=1, N3=1, N4=1, N5=2, and N6=1

366) N1=4, N2=1, N3=1, N4=1, N5=3, and N6=1

367) N1=4, N2=0, N3=0, N4=2, N5=1, and N6=0

368) N1=4, N2=0, N3=0, N4=2, N5=2, and N6=0

369) N1=4, N2=0, N3=0, N4=2, N5=3, and N6=0

370) N1=4, N2=0, N3=0, N4=2, N5=0, and N6=1

371) N1=4, N2=0, N3=0, N4=3, N5=1, and N6=0

372) N1=4, N2=0, N3=0, N4=3, N5=2, and N6=0

373) N1=4, N2=0, N3=0, N4=3, N5=3, and N6=0

374) N1=4, N2=0, N3=0, N4=3, N5=0, and N6=1

375) N1=4, N2=0, N3=0, N4=1, N5=2, and N6=1

376) N1=4, N2=0, N3=0, N4=1, N5=3, and N6=1

377) N1=4, N2=0, N3=0, N4=2, N5=1, and N6=1

378) N1=4, N2=0, N3=0, N4=2, N5=2, and N6=1

379) N1=4, N2=0, N3=0, N4=2, N5=3, and N6=1

380) N1=4, N2=0, N3=0, N4=3, N5=1, and N6=1

381) N1=4, N2=0, N3=0, N4=3, N5=2, and N6=1

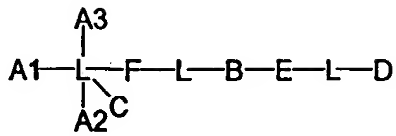
382) N1=4, N2=0, N3=0, N4=3, N5=3, and N6=1

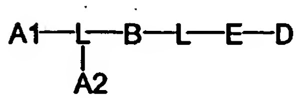
383) N1=4, N2=0, N3=0, N4=1, N5=1, and N6=1

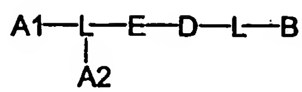
Some preferred embodiments of the present invention and of

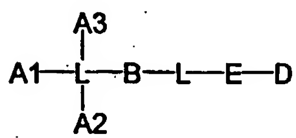
embodiments ET1, ET2, ET3, ET7 and ET8 are shown below and designated as embodiments "ETS 1.X" wherein X= 1, 2, 3, 4, 5, 6...295 and is the number of the structure below:

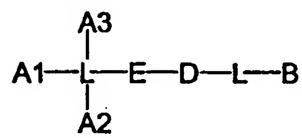
wherein A1,A2, and A3 designate targeting ligands, which may be the same or different; and B, B1, and B2, designate triggers that increase the effector activity PA and may be the same or different, and C designates a masked intracellular transport ligand; and D designates an intracellular trapping ligand; or a masked intracellular trapping ligand; and E, E1, and E2 designate effector agents which may be the same or different, and F designates a trigger that when activated decreases the effector activity PA; and L designates a linker; which may be the same or different from other linkers; and the lines represent the connectivity of the above components:

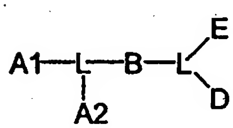
1 

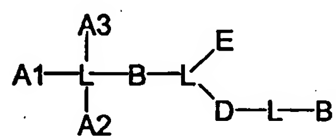
2 

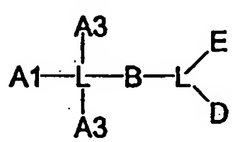
3 

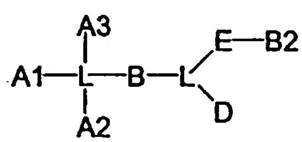
4 

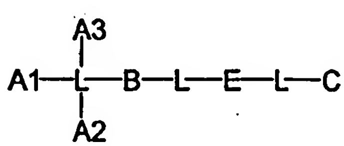
5 

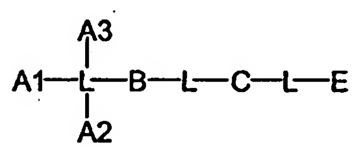
6 

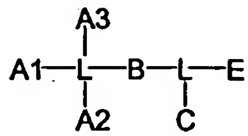
7 

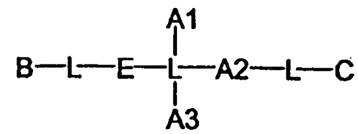
8 

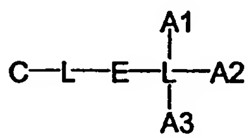
9 

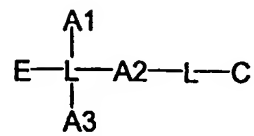
10 

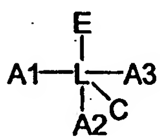
11 

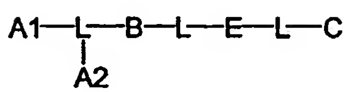
12 

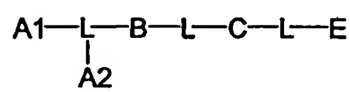
13 

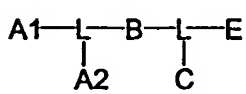
14 

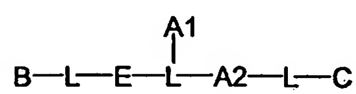
15 

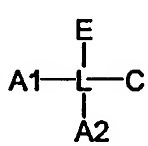
16 

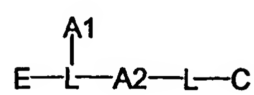
17 

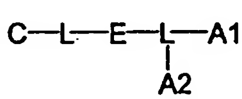
18 

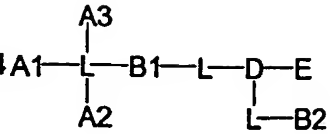
19 

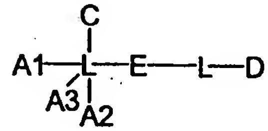
20 

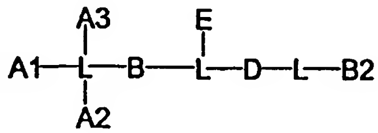
21 

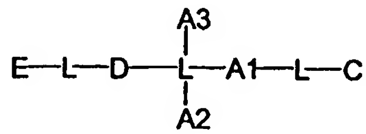
22 

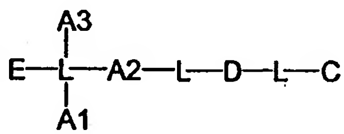
23 

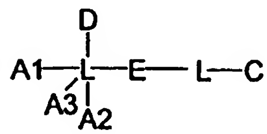
24


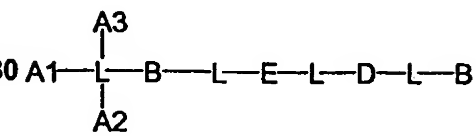
25


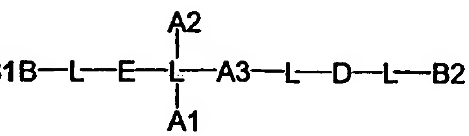
26


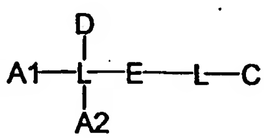
27


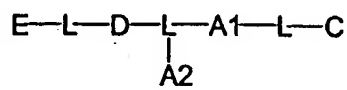
28


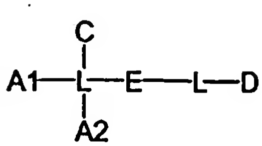
29


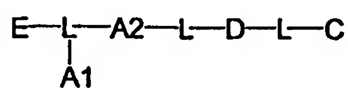
30


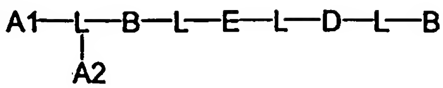
31


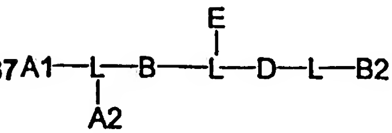
32


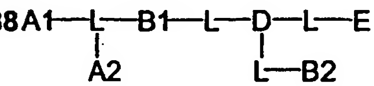
33


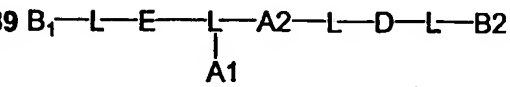
34


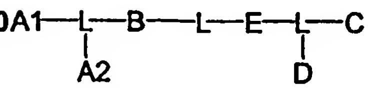
35


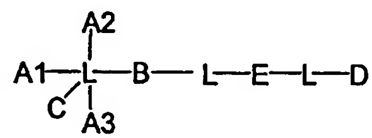
36 

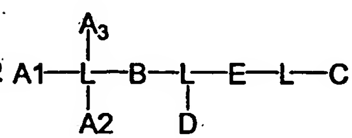
37 

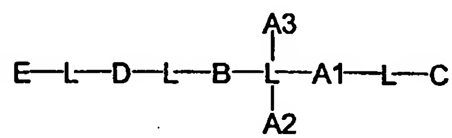
38 

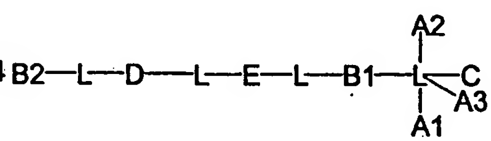
39 

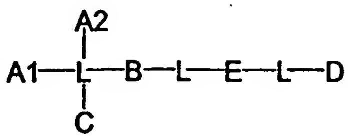
40 

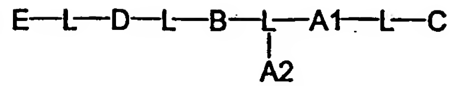
41 

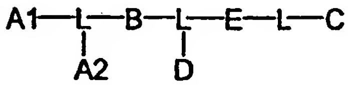
42 

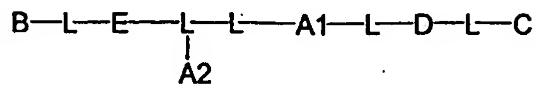
43 

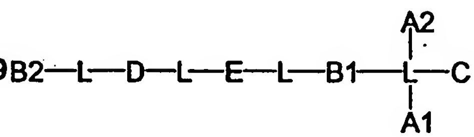
44 

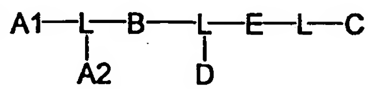
45 

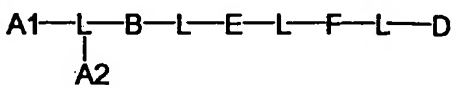
46 

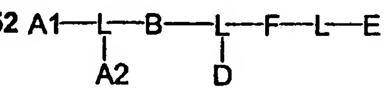
47 

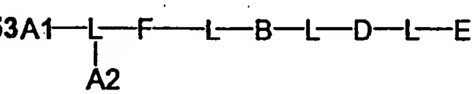
48 

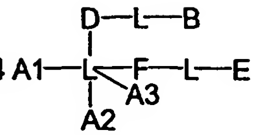
49 

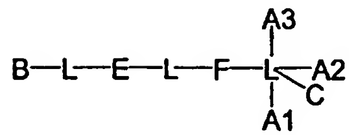
50 

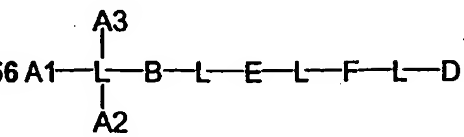
51 

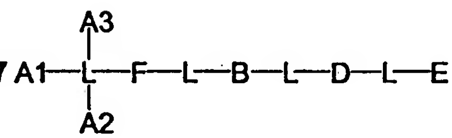
52 

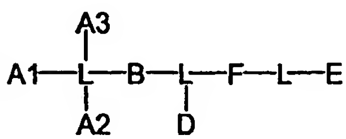
53 

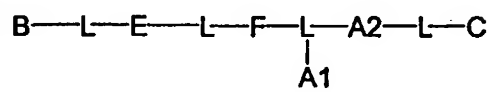
54 

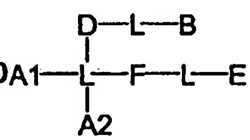
55 

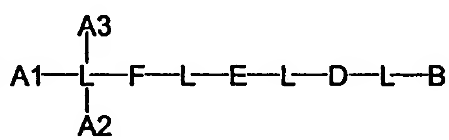
56 

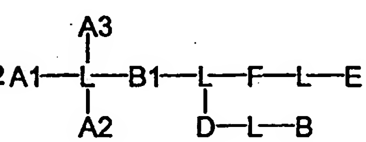
57 

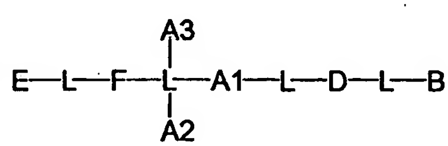
58 

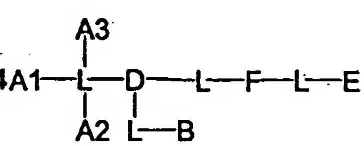
59 

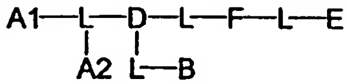
60 

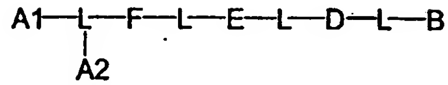
61 

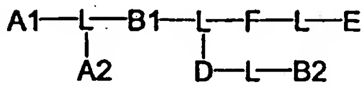
62 

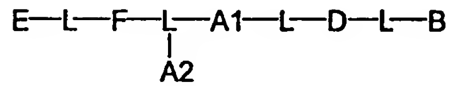
63 

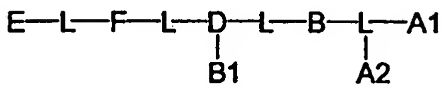
64 

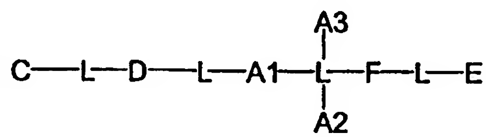
65 

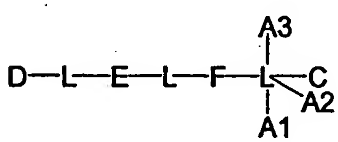
66 

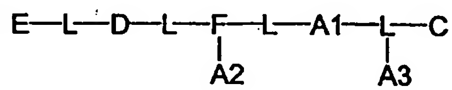
67 

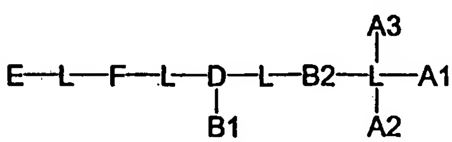
68 

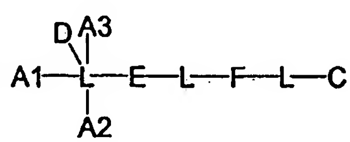
69 

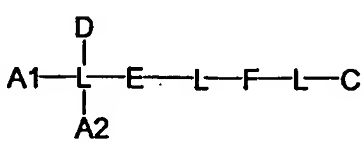
70 

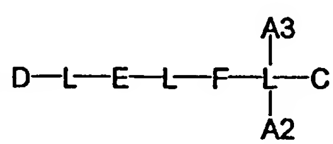
71 

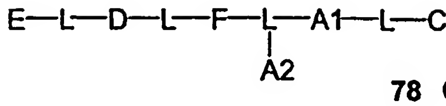
72 

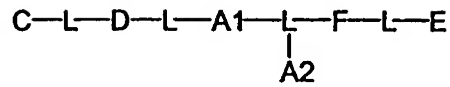
73 

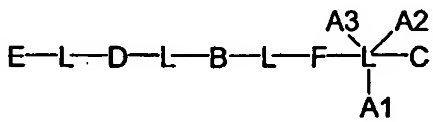
74 

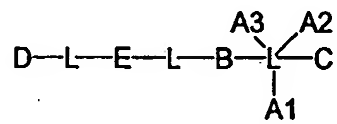
75 

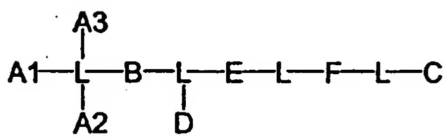
76 

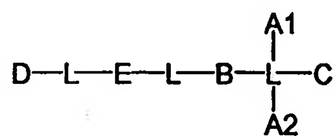
77 

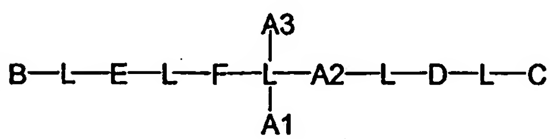
78 

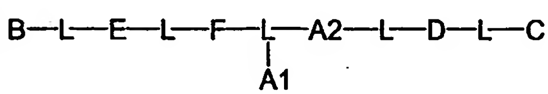
79 

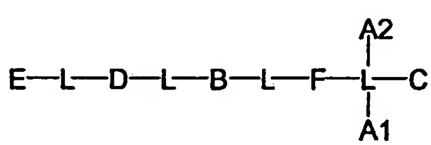
80 

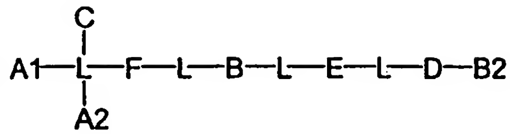
81 

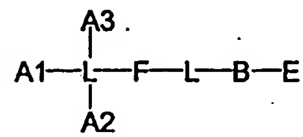
82 

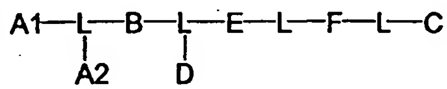
83 

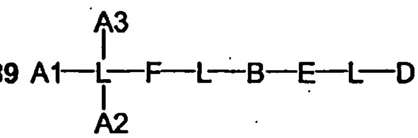
84 

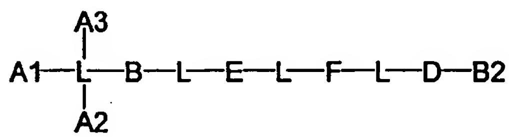
85 

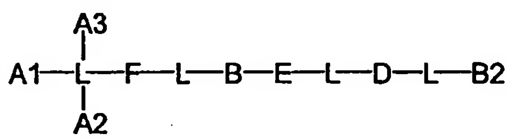
86 

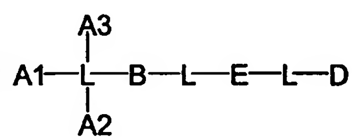
87 

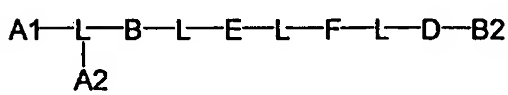
88 

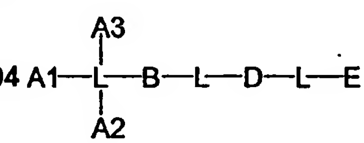
89 

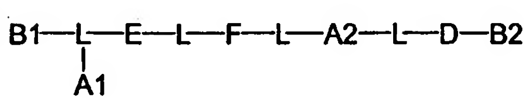
90 

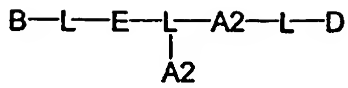
91 

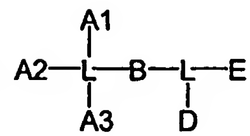
92 

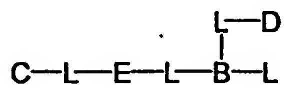
93 

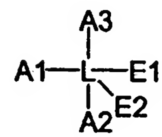
94 

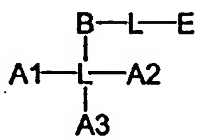
95 

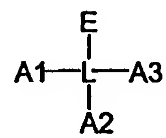
96 

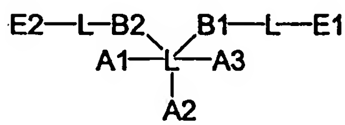
97 

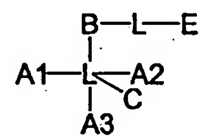
98 

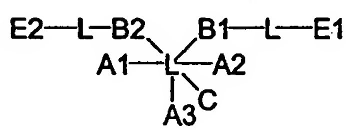
99 

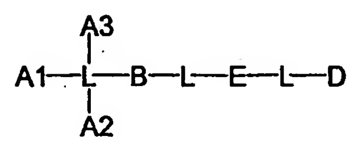
100 

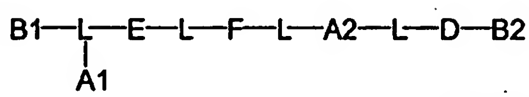
101 

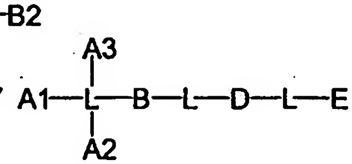
102 

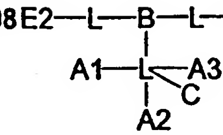
103 

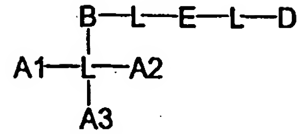
104 

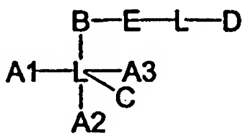
105 

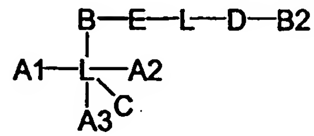
106 

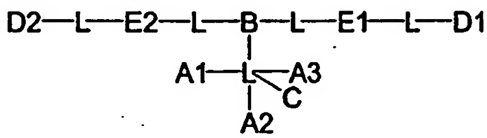
107 

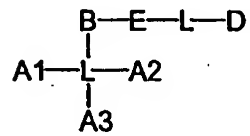
108 

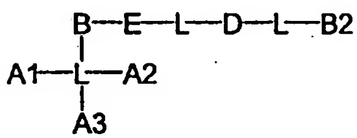
109 

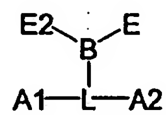
110 

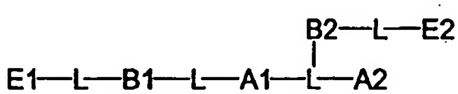
111 

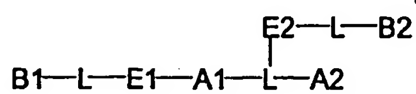
112 

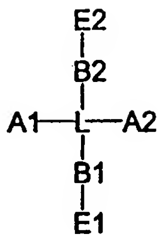
113 

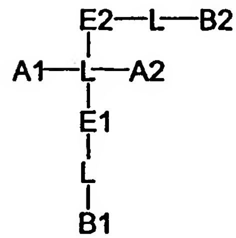
114 

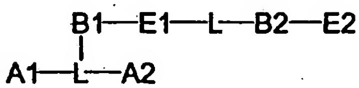
115 

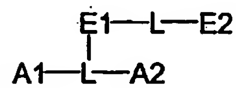
116 

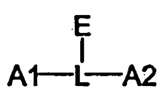
117 

118 

119 

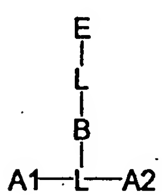
120 

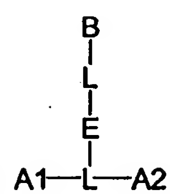
121 

122 

123 A1—L—A2—L—E

124 A1—L—E—L—A2

125 

126 

127 A1—L—A2—L—E—L—B

128
$$\begin{array}{ccccccccc} A1 & — & L & — & E & — & L & — & A2 \\ &&&& | &&&& \\ &&&& L &&&& \\ &&&& | &&&& \\ &&&& B &&&& \end{array}$$

129
$$\begin{array}{ccccccccc} &&&&&& E2 && \\ &&&&&& | && \\ E1 & — & L & — & A1 & — & L & — & A2 \end{array}$$

130
$$\begin{array}{ccccc} && E2 && \\ && | && \\ A1 & — & L & — & A2 \\ && | && \\ && E2 && \end{array}$$

131
$$\begin{array}{ccccccc} && E1 & — & L & — & E2 \\ && | &&&& \\ A1 & — & L & — & A2 && \end{array}$$

132
$$\begin{array}{ccccc} E1 & — & L & — & E2 \\ && | && \\ A1 & — & L & — & A2 \end{array}$$

133 E1—L—A1—L—A2—L—E2

134
$$\begin{array}{ccccccccc} && E1 &&&&&& \\ && | &&&&&& \\ A1 & — & L & — & A2 & — & L & — & E2 \end{array}$$

135
$$\begin{array}{ccccccccc} A1 & — & L & — & A2 & — & L & — & E2 \\ && | &&&&&& \\ && L & — & E1 &&&& \end{array}$$

136
$$\begin{array}{ccccccccc} A1 & — & L & — & A2 & — & L & — & E1 \\ &&&&&& | && \\ &&&&&& E2 && \end{array}$$

137 A1—L—A2—L—E—L—E2

138 E1—L—A1—L—E2—L—A2

139
```
A1—L—E1—L—A2
     |
     L—E2
```

140
```
     E1
     |
A1—L—E2—L—A2
```

141
```
A1—L—E1—L—A2
          |
          E2
```

142 A1—L—E1—L—A2—L—E2

143 E1—L—B1—A1—L—A2—L—E2—B2

144 E1—L—B1—A1—L—A2—L—B2—E2

145
```
     B1—L—E1
     |
A1—L—E2—B2—L—A2
```

146
```
     L—E1
     |
     B1
     |
A1—L—E2—L—A2
```

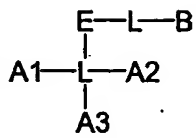
147 A1—L—B1—L—E1—L—E2—B2—L—A2

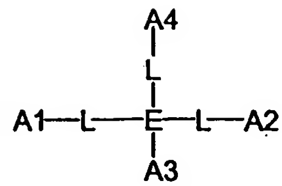
148
```
     E
     |
A1—L—A2—L—A3
```

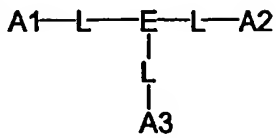
149
```
     E—L—A3
     |
A1—L—A2
```

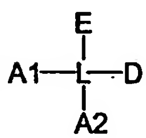
150
```
A1—L—E—L—A2
        |
        A3
```

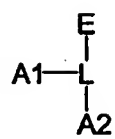
151 A1—L—A2—L—E—L—B—A3

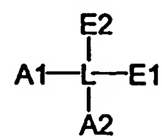
152 

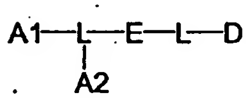
153 

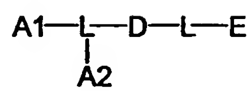
154 

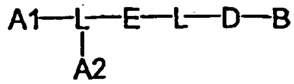
155 

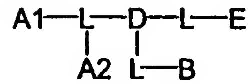
156 

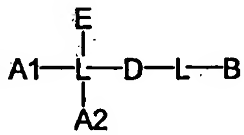
157 

158 

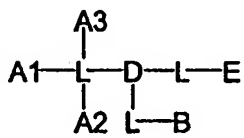
159 

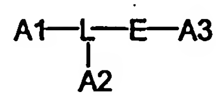
160 

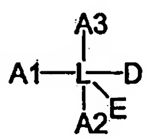
161 

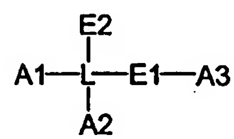
162 

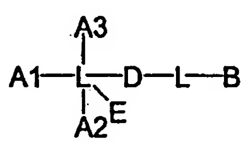
163 E—L—A1—L—A2—L—D—L—B

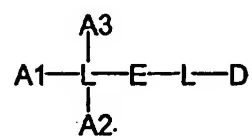
164 

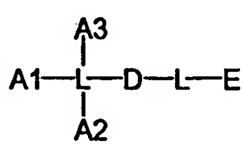
165 

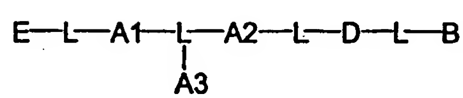
166 

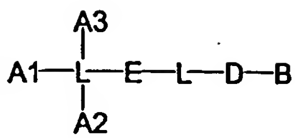
167 

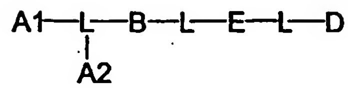
168 

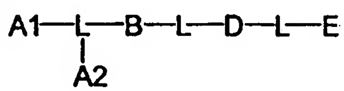
169 

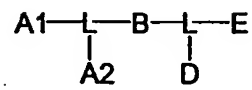
170 

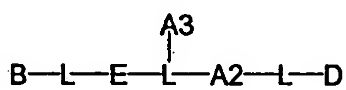
171 

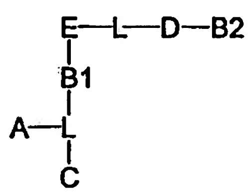
172 

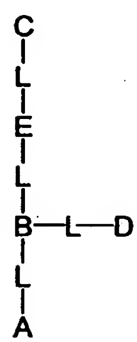
173 

174 

175 

176 

177 

178 

179 A—L—B—L—E—L—F—L—D

180 A—L—F—L—E—L—D—L—E

```
          E
          |
          L
          |
          F
          |
181 A—L—B—L—D
```

182 B—L—E—L—F—L—A—L—D

```
      E
      |
183 F—L—D—L—B
      |
      A
```

```
184 E—L—F—L—D—L—A
            |
            L—B
```

185 B—L—D—L—E—L—F—L—A

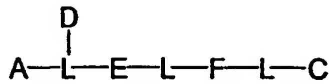
186 E—L—F—L—A—L—D—L—B

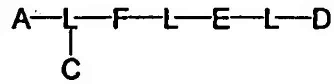
```
            B2—L—D
            |
187 E—L—F—L—B—L—A
```

```
188 E—L—F—L—D—L—B1—L—A
            |
            L—B2
```

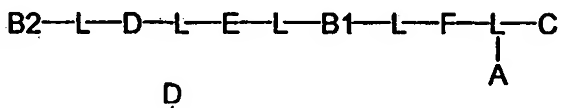
189 B1—L—D—L—F—L—E—B2—L—A

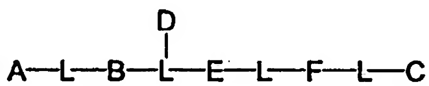
190 B1—L—E—F—L—A—L—D—L—B2

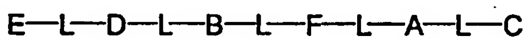
191 

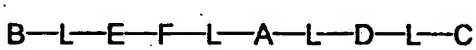
192 

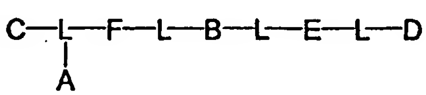
193 

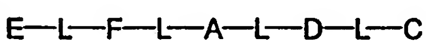
194 

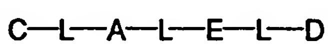
195 

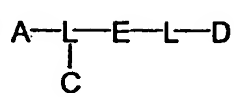
196 

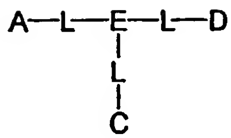
197 

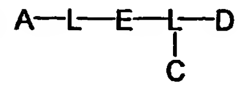
198 

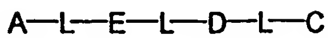
199 

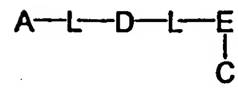
200 

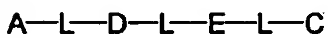
201 

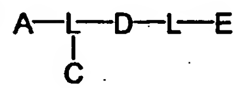
202 

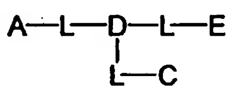
203 

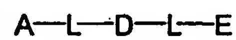
204 

205 

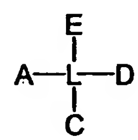
206 

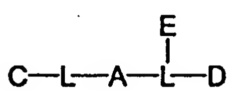
207 

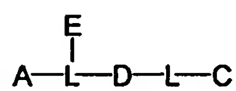
208 

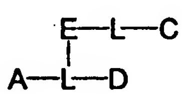
209 

210 

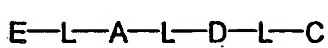
211 

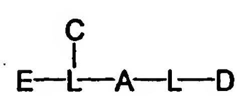
212 

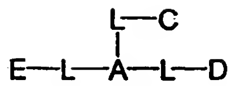
213 

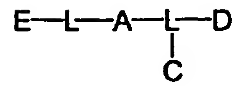
214 

215 

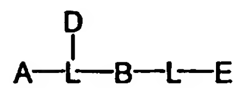
216 

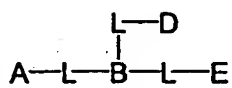
217 

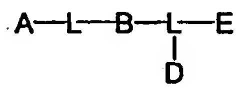
218 

219 

220 D—L—A—L—B—L—E

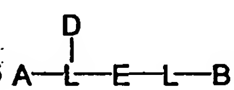
221 

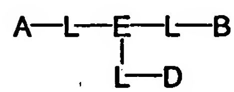
222 

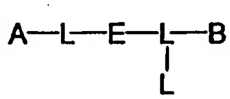
223 

224 A—L—B—L—E—L—D

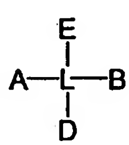
225 D—L—A—L—E—L—B

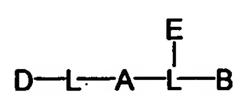
226 

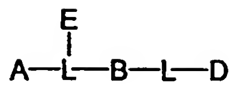
227 

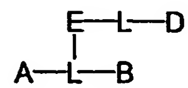
228 

229 A—L—E—L—B—L—C

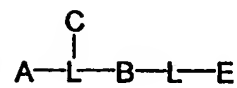
230 

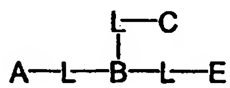
231 

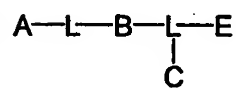
232 

233 

234 

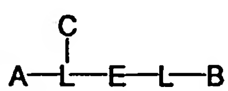
235 

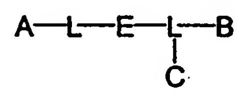
236 

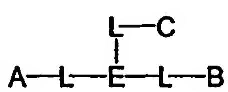
237 

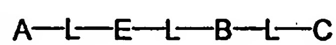
238 

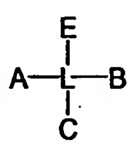
239 

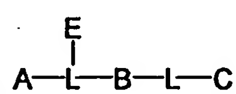
240 

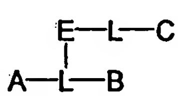
241 

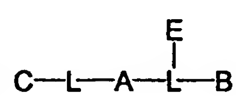
242 

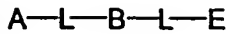
243 

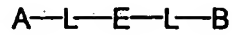
244 

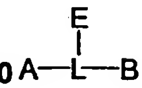
245 

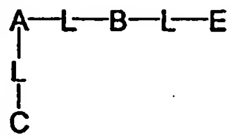
246 

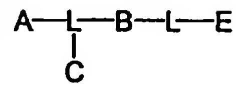
247 

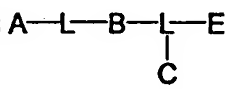
248 

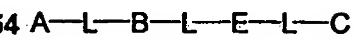
249 

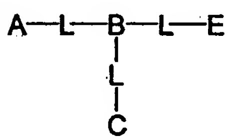
250 

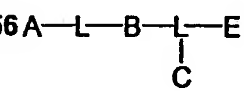
251 

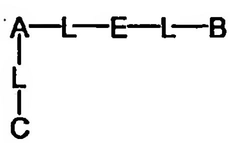
252 

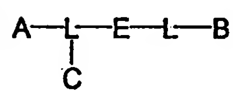
253 

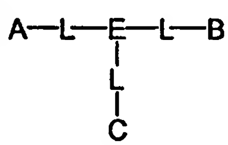
254 

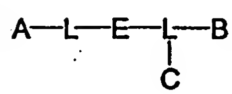
255 

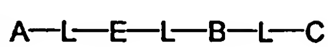
256 

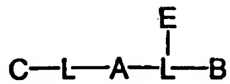
257 

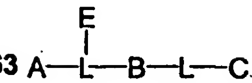
258 

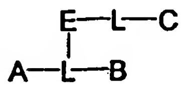
259 

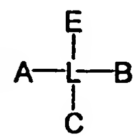
260 

261 

262 

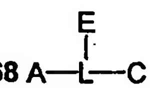
263 

264 

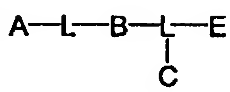
265 

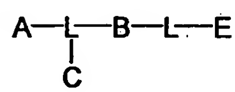
266 A—L—E—L—C

267 A—L—C—L—E

268 

269 E—L—A—L—C

270 

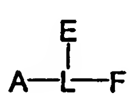
271 

272 A—L—B—L—E—L—C

273 C—L—A—L—B—L—E

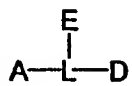
274 A—L—F—L—E

275 A—L—E—L—F

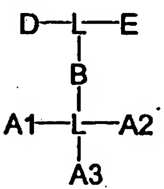
276 

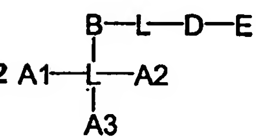
277 A—L—E—L—D

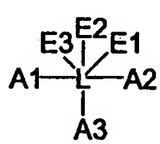
278 A—L—D—L—E

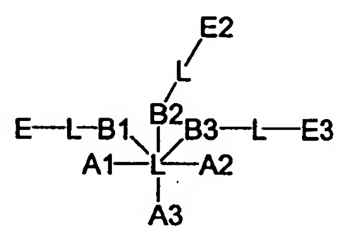
279


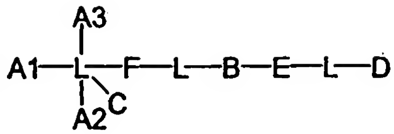
280 E—L—A—L—D

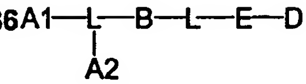
281


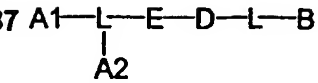
282


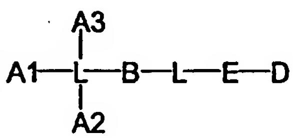
283


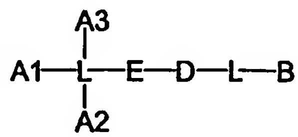
284


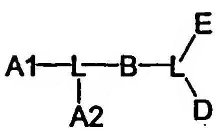
285 

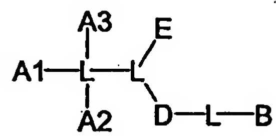
286 

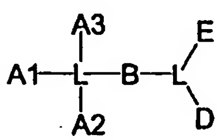
287 

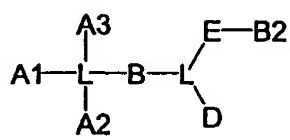
288 

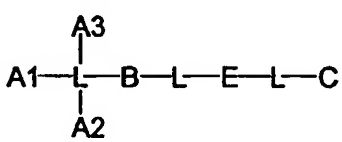
289 

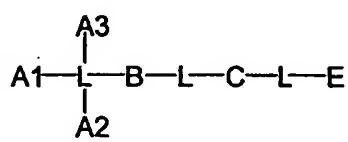
290 

291 

292 

293 

294 

295 

A preferred embodiment comprises ET with one selective targeting ligand at least one masked intracellular transport ligand or where (N1=1 and N2 ≠ 0).

A preferred embodiment comprises ET with one selective targeting ligand at least one detoxification trigger or where (N1=1 and N3 $\neq$ 0).

A preferred embodiment comprises ET with one selective targeting ligand at least one intracellular trapping ligand or masked intracellular trapping ligand or where (N1=1 and N6 $\neq$ 0).

A preferred embodiment comprises ET with one selective targeting ligand at least one trigger or where (N1=1 and N5 $\neq$ 0).

A preferred embodiment comprises ET with two targeting ligands and at least one masked intracellular transport ligand or where (N1=2 and N2 $\neq$ 0).

A preferred embodiment comprises ET with one selective targeting ligand one non-selective targeting ligand.

A preferred embodiment comprises ET with two targeting ligands and at least one detoxification trigger or where (N1=2 and N3 $\neq$ 0).

A preferred embodiment comprises ET with one selective targeting ligand one non-selective targeting ligand.

A preferred embodiment comprises ET with two targeting ligands and at least one intracellular trapping ligand or masked intracellular trapping ligand or where (N1=2 and N6 $\neq$ 0).

A preferred embodiment comprises ET with one selective targeting ligand one non-selective targeting ligand.

A preferred embodiment comprises ET with two targeting ligands and at least one trigger or where (N1=2 and N5 $\neq$ 0).

A preferred embodiment comprises ET with one selective targeting ligand one non-selective targeting ligand.

A preferred embodiment comprises ET with three targeting ligands and at least one masked intracellular transport ligand or where (N1=3 and N2 $\neq$ 0).

A preferred embodiment comprises ET with three targeting ligands and at least one detoxification trigger or where (N1=3 and N3 $\neq$ 0).

A preferred embodiment comprises ET with three targeting ligands and at least one intracellular trapping ligand or masked intracellular trapping ligand or where (N1=3 and N6 $\neq$ 0).

A preferred embodiment comprises ET with three targeting ligands and at least one trigger or where ($N1=3$ and $N5 \neq 0$).

A preferred embodiment comprises ET with 4 targeting ligands or where $N1=4$.

Another preferred embodiment of the present invention is comprised of at least one molecule ET that has been covalently linked to a second molecule that binds to a receptor present in increased amounts at a target cell compared to at a non-target cell; and wherein said second molecule is comprised of a monoclonal antibody, or targeting receptor binding fragment of a monoclonal antibody, or an analog or derivative thereof which bears amino acid sequence similarity to portions of a monoclonal antibody. Also the second molecule coupled to ET can be comprised of a natural protein, or a complex of natural proteins, or a protein, or a naturally occurring polymer that binds to the targeting receptor.

The present invention also comprises (embodiment ET9) a compound ET wherein E is comprised of one or more effector agents designated as E1...En wherein n =1, 2, 3, 4, or 5 or about 5 and wherein these effector agents have pharmacological activity referred to as "PA"; and wherein T is a targeting agent which comprises targeting ligands or targeting ligands and triggers; and wherein T increases the pharmacological activity PA to a target cell compared to non-target cells;

and wherein a targeting ligand is a group that binds selectively to a structure associated with the target referred to as a "targeting receptor";

and wherein a trigger is a group that upon in vivo modification by biomolecules referred to as "triggering agents" becomes activated and modulates the activity of ET;

wherein at the target cells there are present "m" different types of target molecules designated as (p1...pm); at least one of which is present at increased amounts compared to at a non-target cell, and wherein the type of the targeting molecule which is increased on the target cells compared to a non-target cell can be different for a different non-target cell;

and wherein at non-target cells there can be present the same types of target molecules (p1...pm);

and wherein ET is able to "interact with" each of the targeting molecules (p1...pm); wherein the term "interact with" means to bind to a targeting receptor or to have a trigger modified by a triggering agent;

and wherein the number m is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, or 20, or about 20.

In a preferred embodiment (embodiment ET10) the number m is about 2 to 5.

In a preferred embodiment (embodiment ET11) of the present invention, the target is comprised of a tumor, or tumor cell, or components of a tumor, or biomolecules present in the microenvironment of the tumor, or stromal cells present in a tumor, and the effector agent or the pharmacalogical activity PA can evoke or can contribute to tumor cell killing and/or comprises a diagnostic agent.

In a preferred embodiment of the invention and of the embodiments ET1, and ET2, and ET3,and ET4, ET5,and ET6,and ET7,and ET8, and (ET8.X, wherein X=1, 2, 3...383), and ET9, and ET10, and (ETS1.X where X= 1, 2, 3, 4,... 295); designated respectively as embodiments ET12.ET1, and ET12.ET2, and ET12.ET3, and ET12.ET3, and ET12.ET4, and ET12.ET5, and ET12.ET5 and ET12.ET6, and ET12.ET7, and ET12.ET8 and (ET12.ET8.X with X=1,2,3,4...383) and ET12.ET9; and ET12.ET10, and ET12.ET8.X with X= 1, 2, 3, 4, 5...295);

the target is comprised of a tumor, or tumor cell, or components of a tumor, or biomolecules present in the microenvironment of the tumor, or stromal cells present in a tumor, and the effector agent can evoke or can contribute to tumor cell killing and/or comprises a diagnostic agent.

Some preferred embodiments of the invention and of embodiments ET12.ET1, and ET12.ET2, and ET12.ET3, and ET12.ET3, and ET12.ET4, and ET12.ET5, and ET12.ET5 and ET12.ET6, and ET12.ET7; and ET12.ET8 and (ET12.ET8.X with X=1,2,3,4...383) and ET12.ET9; and ET12.ET10, and ET12.ET8.X with X= 1, 2, 3, 4, 5...295); follow:

A preferred embodiment is an anti-cancer drug ET comprised of effector agents that are cytotoxic drugs, and/or radionuclides, and/or immunostimulatory drugs. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that are cytotoxic drugs. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that are radionuclides. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that are cytotoxic drugs that produce synergistic cytotoxicity. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that stimulate the immune system. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that stimulate the innate immune system. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that irreversibly chemically modify one or more tumor components. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that irreversibly chemically modify one or more tumor components that are present in increased amounts in tumor cells or in the microenvironment of tumors compared to vital normal cells. A preferred embodiment is an anti-cancer drug ET comprised of effector agents that potentiates the cytotoxic activity of a second effector agent. A preferred embodiment is an anti-cancer drug ET with an effector agent that comprises an inhibitor to multi-drug transporter proteins. A preferred embodiment is an anti-cancer drug ET with an effector agent that comprises an inhibitor to a membrane protein transporter that faciltates uptake of a nutrient or biomolecule into tumor cells. In a preferred embodiment ET is an anti-cancer drug with an effector agent that comprises an inhibitor to nucleoside transporter proteins.

In a preferred embodiment ET is an anti-cancer drug with targeting ligands that selectively bind to target receptors on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the concentration of the target receptor is greater on the surface of the tumor cell or in the microenvironment of the tumor cell than on the surface or in the microenvironment of normal cells especially vital normal cells.

In a preferred embodiment ET is an anti-cancer drug with an intracellular trapping ligand that selectively binds to one or more intracellular receptors wherein the concentration of the intracellular receptors is greater in tumor cells then in vital normal cells.

In a preferred embodiment ET is an anti-cancer drug with a trigger that increases cytotoxicity of the drug upon in vivo modification and wherein the in vivo modification that activates the trigger is caused by an enzyme or enzymatic activity that is increased at tumor cells or decreased at vital normal cells.

In a preferred embodiment ET is an anti-cancer drug with a trigger that decreases the cytotoxicity of the drug upon in vivo modification and wherein the in vivo modification that activates the trigger is caused by an enzyme or enzymatic activity that is decreased at tumor cells or increased at vital normal cells.

In a preferred embodiment ET is an anti-cancer drug in which the intracellular transport ligand binds to a molecule referred to as a "transporter molecule" to

form a complex and wherein this complex binds to a target cell receptor that actively transports bound ligands into the tumor cell. In a preferred embodiment ET is an anti-cancer drug for which the concentration of transporter molecules is increased at the surface of tumor cells compared to vital normal cells

In a preferred embodiment of the present invention ET is comprised of an anti-cancer drug with two targeting ligands that selectively bind to target receptors on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the concentration of the target receptors is greater on the surface of the tumor cell or in the microenvironment of the tumor cell than on the surface or in the microenvironment of vital normal cells or normal cells. In a preferred embodiment the targeting ligands are the same. In a preferred embodiment the targeting ligands are different and bind to different types of targeting receptors.

In a preferred embodiment ET is an anti-cancer drug with three targeting ligands that selectively bind to target receptors on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the concentration of the target receptors is greater on the surface of the tumor cell or in the microenvironment of the tumor cell than on the surface or in the microenvironment of normal cells or vital normal cells. In a preferred embodiment the targeting ligands are the same. In a preferred embodiment the targeting ligands are different and bind to different types of targeting receptors.

In a preferred embodiment ET is an anti-cancer drug with four targeting ligands that selectively bind to target receptors on the surface of the tumor cell or in the

microenvironment of the tumor cell wherein the concentration of the target receptors is greater on the surface of the tumor cell or in the microenvironment of the tumor cell than on the surface or in the microenvironment of normal cells or vital normal cells. In a preferred embodiment the targeting ligands are the same. In a preferred embodiment the targeting ligands are different and bind to different types of targeting receptors.

In another preferred embodiment ET is an anti-cancer drug comprised of a compound with two or more targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell wherein the target has an increased amount of that target receptor compared to a non-target cell that binds to a second targeting ligand of the compound. A preferred embodiment of this embodiment comprises a compound with two different targeting ligands that bind to two different targeting receptors. Another preferred embodiment of this embodiment comprises a compound with three different targeting ligands that bind to three different targeting receptors. Another preferred embodiment of this embodiment comprises a compound with four different targeting ligands that bind to four different targeting receptors. In a preferred embodiment the drug binds to at most one type of receptor present on normal cells.

In another preferred embodiment the anti-cancer compound ET is comprised of one tumor-selective targeting ligand at least one masked intracellular transport ligand or where ($N1=1$ and $N2 \neq 0$).

In another preferred embodiment the anti-cancer compound ET is comprised of one tumor-selective targeting ligand at least one detoxification trigger or where ($N1=1$ and $N3 \neq 0$).

In another preferred embodiment the anti-cancer compound ET is comprised of one tumor-selective targeting ligand at least one intracellular trapping ligand or masked intracellular trapping ligand or where ($N1=1$ and $N6 \neq 0$).

In another preferred embodiment the anti-cancer compound ET is comprised of one tumor-selective targeting ligand at least one trigger or where ($N1=1$ and $N5 \neq 0$).

In another preferred embodiment the anti-cancer compound ET is comprised of two targeting ligands and at least one masked intracellular transport ligand or where ($N1=2$ and $N2 \neq 0$). In a preferred embodiment of this the anti-cancer compound ET is comprised of one selective targeting ligand one non-selective targeting ligand. In another embodiment of this both targeting ligands are tumor-selective.

In another preferred embodiment the anti-cancer compound ET is comprised of two targeting ligands and at least one detoxification trigger or where ($N1=2$ and $N3 \neq 0$). In a preferred embodiment of this the anti-cancer compound ET is comprised of one selective targeting ligand one non-selective targeting ligand. In another embodiment of this, both targeting ligands are tumor-selective.

In another preferred embodiment the anti-cancer compound ET is comprised of two targeting ligands and at least one intracellular trapping ligand or masked intracellular trapping ligand or where (N1=2 and N6 $\neq$ 0). In a preferred embodiment of this the anti-cancer compound ET is comprised of one selective targeting ligand one non-selective targeting ligand. In another embodiment of this, both targeting ligands are tumor-selective.

In another preferred embodiment the anti-cancer compound ET is comprised of two targeting ligands and at least one trigger or where (N1=2 and N5 $\neq$ 0). In a preferred embodiment of this the anti-cancer compound ET is comprised of one selective targeting ligand one non-selective targeting ligand. In another embodiment of this, both targeting ligands are tumor-selective.

In another preferred embodiment the anti-cancer compound ET is comprised of three targeting ligands and at least one masked intracellular transport ligand or where (N1=3 and N2 $\neq$ 0).

In another preferred embodiment the anti-cancer compound ET is comprised of three targeting ligands and at least one detoxification trigger or where (N1=3 and N3 $\neq$ 0).

In another preferred embodiment the anti-cancer compound ET is comprised of three targeting ligands and at least one intracellular trapping ligand or masked intracellular trapping ligand or where (N1=3 and N6 $\neq$ 0).

In another preferred embodiment the anti-cancer compound ET is comprised of three targeting ligands and at least one trigger or where (N1=3 and N5 $\neq$ 0).

In another preferred embodiment the anti-cancer compound ET is comprised of 4 targeting ligands or where (N1=4).

In another preferred embodiment the anti-cancer compound ET is comprised of two identical tumor-selective targeting ligands and one effector agent or where N1=2, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this, the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide.

In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two different tumor-selective targeting ligands and one effector agent or where N1=2, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two identical tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent or where N1=2, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two different tumor-selective targeting ligands that bind to different tumor-selective receptors and one effector agent and one trigger that increases the toxicity of the effector agent or where N1=2, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two identical tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector and one masked intracellular transporter ligand; or where N1=2, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In

another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two different tumor-selective targeting ligands that bind to two different tumor-selective receptors and one effector agent and one trigger that increases the toxicity of the effector and one masked intracellular transporter ligand; or where N1=2, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand, and one intracellular trapping ligand or one masked intracellular trapping ligand or where N1=2, and N2=1, and N3=0, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of

a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent, and one trigger that decrease the toxicity of the effector agent, and one masked intracellular transport ligand or where N1=2, and N2=1, and N3=1, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of two different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent, and one trigger that

decrease the toxicity of the effector agent, and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand. or where N1=2, and N2=1, and N3=1, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and one effector agent or where N1=3, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and one effector agent and one trigger that increases the toxicity of the effector agent, or where N1=3, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transporter ligand or where N1=3, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor

components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, or where N1=3, and N2=1, and N3=0, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, and one trigger that decreases the toxicity of the effector agent or where N1=3, and N2=1, and

N3=1, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and two effector agents or where N1=3, and N2=0, and N3=0, and N4=2, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and two effector agents and two triggers, or where N1=3, and N2=0,

and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and two effector agents, and two triggers, and one masked intracellular transport ligand or where N1=3, and N2=1, and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of three different tumor-selective targeting ligands to three different targeting receptors and two effector agents, and two triggers, and one masked intracellular transport ligand an intracellular trapping ligand or a masked intracellular trapping ligand, or where N1=3, and N2=1, and N3=0, and N4=2, and N5=2, and N6=1. In a preferred embodiment of this, the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and one effector agent or where N1=4, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In

another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and one effector agent and one trigger that increases the toxicity of the effector agent, or where N1=4, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transporter ligand or where N1=4, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In

another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, or where N1=4, and N2=1, and N3=0, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping

ligand or masked intracellular trapping ligand, and one trigger that decreases the toxicity of the effector agent or where N1=4, and N2=1, and N3=1, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and two effector agents or where N1=4, and N2=0, and N3=0, and N4=2, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and two effector agents and two triggers, or where N1=4, and N2=0, and N3=0, and N4=2, and N5=2, and N6=0.

In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and two effector agents, and two triggers, and one masked intracellular transport ligand or where N1=4, and N2=1, and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of

an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of four different tumor-selective targeting ligands to four different target receptors and two effector agents, and two triggers, and one masked intracellular transport ligand an intracellular trapping ligand or a masked intracellular trapping ligand, or where N1=4, and N2=1, and N3=0, and N4=2, and N5=2, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective targeting ligands that bind to different targeting ligands and one effector agent or where N1=2, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands that bind to different targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective

targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent or where N1=2, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective targeting ligands that bind to different targeting ligands and one effector agent and one trigger and one masked intracellular transporter ligand or where N1=2, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the

effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective targeting ligands that bind to different targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand, and one intracellular trapping ligand or one masked intracellular trapping ligand or where N1=2, and N2=1, and N3=0, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective targeting ligands that bind to different targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent, and one trigger that decreases the toxicity of the effector agent, and one masked intracellular transport ligand or where N1=2, and N2=1, and N3=1, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount

of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective targeting ligands that bind to different targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent, and one trigger that decreases the toxicity of the effector agent, and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand or where N1=2, and N2=1, and N3=1, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with two targeting ligands wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein ET is comprised of two different tumor-selective targeting ligands that bind to different targeting ligands and two tumor-selective targeting ligands and two effector agents or

where N1=2 and N4=2. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound. In a preferred embodiment of this the effector agent is a

cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of one effector agent or where N1=3, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another

embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of one effector agent and one trigger that increases the toxicity of the effector agent, or where N1=3, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the

tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transporter ligand or where N1=3, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, or where N1=3, and N2=1, and N3=0, and

N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, and one trigger that decreases the toxicity of the effector agent or where N1=3, and N2=1, and N3=1, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the

effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of two effector agents or where N1=3, and N2=0, and N3=0, and N4=2, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of two effector agents and two triggers, or where N1=3, and N2=0, and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of two

effector agents, and two triggers, and one masked intracellular transport ligand or where N1=3, and N2=1, and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with three different targeting ligands that bind to three different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of two effector agents, and two triggers, and one masked intracellular transport ligand an intracellular trapping ligand or a masked intracellular trapping ligand, or where N1=3, and N2=1, and N3=0, and N4=2, and N5=2, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised

of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and one effector agent or where N1=4, and N2=0, and N3=0, and N4=1, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune

system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent, or where N1=4, and N2=0, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transporter ligand or where N1=4, and N2=1, and N3=0, and N4=1, and N5=1, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor

compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, or where N1=4, and N2=1, and N3=0, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and one effector agent and one trigger that increases the toxicity of the effector agent and one masked intracellular

transport ligand one intracellular trapping ligand or masked intracellular trapping ligand, and one trigger that decreases the toxicity of the effector agent or where N1=4, and N2=1, and N3=1, and N4=1, and N5=1, and N6=1. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and two effector agents or where N1=4, and N2=0, and N3=0, and N4=2, and N5=0, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an

effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and two effector agents and two triggers, or where N1=4, and N2=0, and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and two effector agents, and two triggers, and one masked intracellular transport ligand or where N1=4, and N2=1, and N3=0, and N4=2, and N5=2, and N6=0. In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment the anti-cancer compound ET is comprised of a compound with four different targeting ligands that bind to four different target receptors and wherein at least one of the targeting ligands binds to a target receptor on the surface of the tumor cell or in the microenvironment of the tumor cell wherein the tumor has an increased amount of that target receptor compared to a normal cell or a vital normal cell that binds to a second targeting

ligand of the compound; and wherein the compound is comprised of four different tumor-selective targeting ligands and two effector agents, and two triggers, and one masked intracellular transport ligand an intracellular trapping ligand or a masked intracellular trapping ligand, or where N1=4, and N2=1, and N3=0, and N4=2, and N5=2, and N6=1.

In a preferred embodiment of this the effector agent is a cytotoxic drug. In another embodiment of this, the effector agent is comprised of a radionuclide. In another embodiment of this, the effector agent is comprised of a drug that stimulates the immune system. In another embodiment of this, the effector agent is comprised of an effector agent that irreversibly chemically modifies one or more tumor components. In another embodiment of this, the effector agent is comprised of an inhibitor to multi-drug transporter proteins. In another embodiment of this, the effector agent is comprised of an inhibitor to nucleoside transporter proteins.

In another preferred embodiment, ET is an anti-cancer drug comprised of a compound with two or more targeting ligands that binds to a tumor cell with an affinity that is greater than a normal cell presenting a target receptor(s) that bind to the targeting ligands of said compound. In preferred embodiments the above mentioned binding affinity to the tumor cell is at least about 2-5 times greater, or at least about 5-10 times greater, or at least about 10-50 times greater, or at least about 50-500 times greater, or at least about 500-5,000 times greater, or at least about 5,000-50,000 times greater, or at least about 50,000-1,000,000 times greater or more than 1 million times greater than to a normal cell or to a

vital normal cell. In a preferred embodiment the compound has three different targeting ligands. In another preferred embodiment the compound has 4 different targeting ligands.

In another preferred embodiment ET is an anti-cancer drug with binding affinity to tumor cells that is approximately the same as to populations of normal cells. However, said population of normal cells have decreased sensitivity to the toxic effects of the effector agent because said normal cells have decreased levels of an intracellular trapping receptor, or decreased sensitivity to the effector agent, or decreased levels of a specific protein necessary for neoantigen formation, or by virtue of said normal cells being located in the body at a site, such as the brain, where the drug ET cannot penetrate.

In another preferred embodiment the anti-cancer drug ET is comprised of:

I. N1 targeting ligands, which can differ;
II. N2 masked intracellular transport ligands which can differ;
III. N3 triggers, which can differ, designated "detoxification triggers" wherein activation of the trigger decreases the toxicity of the drug;
IV. N4 effector agents which can differ;
V. N5 triggers which can differ, wherein activation of the trigger increases the toxicity of the drug;
VI. N6 intracellular trapping ligands or masked intracellular trapping ligands, which can differ;

and wherein:

N1 =1, 2, 3, or 4, or about 4; N2 =0, 1, or 2, or about 2;

N3 =0, 1, or 2, or about 2; N4 =1, 2, or 3, or about 3;

N5 =0, 1, 2,or 3, or about 3; N6 =1, 2, or 3, or about 3;

And, wherein ET evokes a greater toxicity to a tumor cell compared to a non-tumor cell or a vital normal cell and wherein this increased antitumor selectivity is due to functional cooperation between the components of ET and not due to any single component of ET.

In a preferred embodiment of the invention and of the embodiments ET12.ET1, and ET12.ET2, and ET12.ET3, and ET12.ET3, and ET12.ET4, and ET12.ET5, and ET12.ET5 and ET12.ET6, and ET12.ET7, and ET12.ET8 and (ET12.ET8.X with X=1,2,3,4...383) and ET12.ET9; and ET12.ET10, and ET12.ET8.X with X= 1, 2, 3, 4, 5...X);

the compound ET is comprised of an anti-cancer drug with at least one targeting ligand that binds to a target receptor selected from the following list:

1.) a cathepsin type protease

2.) a collagenase

3.) a gelatinase

4.) a matrix metalloproteinase

5.) a membrane type matrix metalloproteinase

6.) alpha v beta 3 integrin

7.) bombesin /gastrin releasing peptide receptors

8.) cathepsin B

9.) cathepsin D

10.) cathepsin K

11.) cathepsin L

12.) cathepsin O

13.) fibroblast activation protein

14.) folate binding receptors

15.) gastrin/cholecystokinin type B receptor

16.) glutamate carboxypeptidase II or (PSMA)

17.) guanidinobenzoatase

18.) laminin receptor

19.) matrilysin or

20.) matripase

21.) melanocyte stimulating hormone receptor

22.) nitrobenzylthioinosine-binding receptors

23.) norepenephrine transporters

24.) nucleoside transporter proteins

25.) peripheral benzodiazepam binding receptors

26.) plasmin

27.) seprase

28.) sigma receptors

29.) somatostatin receptors

30.) stromelysin 3

31.) trypsin

32.) urokinase

33.) MMP 1

34.) MMP 2

35.) MMP 3

36.) MMP 7

37.) MMP 9

38.) Membrane type matrix metalloproteinase I

39.) MMP 12

40.) MMP 13

In a preferred embodiment of the present invention ET is comprised of an anti-cancer drug with two targeting ligands for receptors that are increased on a tumor cell compared to a normal cell wherein at least one of the targeting ligands binds to a receptor selected from the list given above.

In a preferred embodiment ET is comprised of an anti-cancer drug with 2 targeting ligands that bind to receptors selected from the above list. In a preferred embodiment these receptors are the same. In a preferred embodiment these receptors are different and bind to different receptors.

In a preferred embodiment ET is comprised of an anti-cancer drug with 3 targeting ligands that bind to receptors selected from the above list. In a preferred embodiment these receptors are the same. In a preferred embodiment these receptors are different and bind to different receptors.

In a preferred embodiment ET is comprised of an anti-cancer drug with 4 targeting ligands that bind to receptors selected from the above list. In a preferred embodiment these receptors are the same. In a preferred embodiment these receptors are different and bind to different receptors.

In a preferred embodiment of the anti-cancer drug ET the targeting ligands are selected to bind to targeting receptors that are enriched on tumor cells or in the microenvironment of tumor cells.

MECHANISM OF ACTION

A preferred embodiment of the invention comprises an anti-cancer drug comprised of 2 to n targeting ligands designated as "A1", and "A2",.... "An" that are connected by a linker designated as "L", and wherein "An" refers to a targeting ligand that can bind to a targeting receptor designated "an" that is enriched on the surface of or in the microenvironment of the target and to which is also attached aone or more cytotoxic cytotoxic agents.

The targeting ligand-target receptor complex a1-A1-L-A2-a2 can be stabilized by the binding energy of both the A1-a1 and A2-a2 interactions which can result in extraordinary affinity of E-T to the target cell. By this mechanism super high affinity (essentially irreversible) targeting is possible provided that both A1-a1 and A2-a2 are high affinity bindings. Doubling the decrease in standard free energy for a reaction squares the equilibrium constant. Although entropic factors can intervene to preclude the addition of a second receptor site from actually doubling the standard free energy change, the impact on the equilibrium constant (binding affinity) can be enormous. Targeting affinity exceeding that seen with monoclonal antibodies can be achieved with low molecular weight compounds. An important consequence of this type of multivalent binding is multifactorial targeting. Super high affinity binding can occur only if the target cell has both targeting receptors a1 and a2. The higher the affinity the lower the

drug concentration required to bind the drug to the target cell. Accordingly, at sufficiently low concentrations the drug can bind almost exclusively to target cells that jointly express both a1 and a2.

The relationship between increased binding affinity and multisite binding is a consequence of the most basic laws of thermodynamics and is exemplified by the properties of antibodies, peptabodies, certain drug dimers which display multisite binding affinity up to a million times greater than with single site binding. The following reference relates to this subject matter: Kaufman E.N.; Jain R.K., "Effect of Bivalent Interaction upon Apparent Antibody Affinity: Experimental Confirmation of Theory Using Fluorescence Photobleaching and Implications for Antibody Binding Assays," *Cancer Research,* 52:4157-4167 (1992); Terskikh A.V., et al., "'Peptabody': A New Type of High Avidity Binding Protein," *Proc Natl Acad Sci USA,* 94:1663-1668 (1997); Hubble J., "A Model of multivalent Ligand-receptor Equilibria which Explains the Effect of Multivalent Binding Inhibitors," *Molecular Immunology,* 36:13-18 (1999); Pagé D.; René Roy, "Synthesis and Biological Properties of Mannosylated Starburst Poly(amidoamine) Dendrimers," *Bioconjugate Chem,* 8:714-723 (1997); Calas M., et al., "Antimalarial Activity of Compounds Interfering with *Plasmodium falciparum* Phospholipid Metabolism: Comparison between Mono- and Bisquaternary Ammonium Salts," *J Med Chem,* 43:505-516 (2000); Kramer R.H.; Karpen J.W., "Spanning Binding Sites on Allosteric Proteins with Polymer-linked Ligand Dimers," *Nature,* 395:710-713 (1998); Fan E., et al., "High-Affinity Pentavalent Ligands of *Escherichia coli* Heat-Labile Enterotoxin by Modular Structure-Based Design," *J Am Chem Soc,* 122:2663-2664 (2000); Blaustein

R.O., et al., "Tethered Blockers as Molecular 'Tape Measures' for a Voltage-gated K+ Channel," *Nature Structure Biol*, 7(4):309-311 (2000); Riley A. M.; Potter B.V.L., "Poly(ethylene glycol)-Linked Dimers of D-*myo*-inositol 1,4,5-trisphosphate," *Chem Commun*, 983-984 (2000); Mammen M., et al., "Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors," *Angew Chem Int Ed*, 37:2754-2794 (1998); Johnson D.L., et al., "Amino-terminal Dimerization of an Erythropoietin Mimetic Peptide Results in Increased Erythropoietic Activity," *Chem Biol*, 4:939-950 (1997), the contents of which are incorporated herein by reference in their entirety.

If A1 and A2 are identical and the target site a1 is present at sufficient density on the target cell then drugs incorporating this structure can induce crosslinking of the cell receptors. Many membrane associated proteins are highly mobile within the surface of the cell membrane. The binding energy of the drug to the cell can also be substantially increased which can translate into a markedly increased affinity and potency of targeting. If the affinity of A1 to its target site is high then the crosslinked form can be essentially irreversible. Crosslinking of the receptors can also enhance cellular uptake by triggering endocytosis. The following reference relates to this subject matter: York S.J. et al., "The Rate of Internalization of the Mannose 6-Phosphate/Insulin-like Growth Factor II Receptor is Enhanced by Multivalent Ligand Binding," *J Biol Chem*, 274(2):1164-1171 (1999), the contents of which are incorporated herein by reference in their entirety.

The rate of crosslinking can be a function of the square of the receptor concentration. For example, if a tumor cell has 10 times more target sites a1 than normal cells then the tumor cell can form crosslinked receptors at a rate 100 times faster (to a first approximation) than the normal cells. A prerequisite for the successful application of this class of compounds is that the receptor density on the target cell be sufficiently high to allow crosslinking to occur at a meaningful rate. The linker length can be selected to optimize crosslinking capacity.

In the embodiment where A1 and A2 are different, the rate of crosslinking and essentially irreversible binding of the prodrug to the cell can be a function of the product of the concentration of the receptor target sites a1 and a2. For example, if a tumor cell has 10 times more a1 and 30 times more a2 than normal cells then the tumor cell can form crosslinked receptors at a rate approximately 300 times faster than the normal cells. If the product of the concentration is too low then the magnitude of the avidity enhancement can be minimal. Accordingly, if a1 is a target receptor, which is present only at very low concentrations, then a2 can be selected to be a target receptor, which is present at high concentrations.

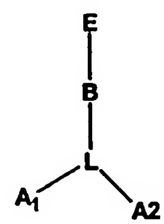
The embodiment, in which a1 is enriched on the target cell and a2 is present on target and normal cells at equal concentrations, also has useful applications. For example, if a1 is a cell membrane protein, which is poorly internalized then a drug complex coupled to a1 can fail to enter the target cell. However, if a2 is a cell membrane protein that undergoes facile endocytosis then crosslinked complex can be transported into the cell with increased efficiency.

A2 can also serve to localize the drug to the cell membrane. For example, if A2 is a simple fatty acid it can partition into the cell membrane in a nonspecific fashion. Nonetheless, this can contribute significantly to the binding energy of the drug to the cell and markedly increase overall target cell affinity. The transfer of a fatty acid chain from solution to the lipid phase of the membrane is expected to be a much slower process then the binding of typical high affinty ligand-receptors that are often under diffusion control. Since the equilbrium constant is the ratio of the forward and backward reaction rates (rate of solvation / rate of desolvation), the rate at which the fatty acid group desolvates from the cell membrane can be even slower which can contribute to the retention of the targeted drug to the target cell. Accordingly, the use of a nonspecific group which binds with relatively low energy, and has minimal entropic requirements, in conjunction with a target selective high affinity ligand can markedly enhance targeting effectiveness. It can be noted that the drugs are designed for use in the nanomolar to picomolar range orders of magnitude below the critical micelle concentration.

In another preferred embodiment the drug has three target selective ligands A1, A2, and A3. Drugs of this type can bind with high affinity to target cells that express all three or any combination of two of the receptors (a1, a2, a3 or a1, a2, or a2, a3 or, a1, a3). The advantage of having three receptors is that loss of one receptor is unlikely to confer the tumor resistance to the drug.

In a preferred embodiment, A1 and A2 are selected so as to bind to target sites that are enriched on tumor cells compared to normal cells. For example, A1 or A2 can bind to a receptor, structural component, or enzyme located on the tumor cell surface or to an enzyme that binds to the cell surface.

A preferred embodiment of the invention and of embodiment ET8 has the structure shown below:



Wherein A1 and A2 are tumor-selective targeting ligands, and the L are linkers and B is a trigger that when activated frees the effector agent E from the remainder of the drug ; and wherein E is a cytotoxin.

Drugs of this class feature two tumor specific high affinity binding ligands covalently coupled via a linker designed to allow both A1 and A2 to interact with receptors a1 and a2 on the tumor cell surface. A toxic moiety is coupled covalently to the linker via a functionality which has a trigger mechanism that when activated releases the toxin. The requirements for the trigger functionality differ depending upon the nature of the toxin to be delivered and the rate of cellular uptake of E-T. If the free toxin is readily internalized by the target cells then a trigger can be activated by extracellular or ultracellular enzymes or chemical processes. In a preferred embodiment, the trigger can be activated by an enzyme that is enriched in the tumor microenvironment. If the free toxin is

poorly taken up by tumor cells, then a trigger that is preferentially activated inside cells can be used to free the drug intracellularly. This can be achieved by employing a trigger that is activated by intracellular enzymes. Alternatively, the trigger can be activated by extracelluar enzymes or by spontaneous chemical processes provided that a time delay mechanism is incorporated which allows sufficient time between trigger activation and toxin release for the drug ligand complex E-T to be internalized. Finally, in circumstances where the toxin is effective extracellullary (or intracellularly when still attached to the targeting ligands), the trigger can be omitted entirely.

In a preferred embodiment the trigger can be activated by an enzyme that is delivered to the target cell via independently selective mechanisms. There have been intense efforts towards the development of tumor-selective antibodies coupled to enzymes to selectively activate prodrugs. A significant limitation with Antibody Directed Enzyme Prodrug Therapy (ADEPT), and related approaches is the requirement that for the targeted enzyme to efficiently activate the prodrug, the prodrug can be given at a concentration near the Michaelis Menton constant (Km) for the enzyme substrate interaction which is generally micromolar. Since all drugs are expected to have multiple pathways of metabolism, prodrug activation by non-targeted enzyme mechanisms can result in dose limiting toxicity. The following reference relates to this subject matter: Bagshawe K.D., "ADEPT and Related Concepts," *Cell Biophys,* 24-25:83-91 (1994); Syrigos K.N.; Epenetos A.A., "Antibody Directed Enzyme Prodrug Therapy (ADEPT): A Review of the Experimental and Clinical Considerations," *Anti-cancer Res,* 19(1A):605-13 (1999); Bagshawe K.D., "Antibody-Directed

Enzyme Prodrug Therapy for Cancer: Its Theoretical Basis and Application," *Mol Med Today,* 1(9):424-31 (1995); Melton R.G.; Sherwood R.F., "Antibody-Enzyme Conjugates for Cancer Therapy," *J Natl Cancer Inst,* 88(3-4):153-65 (1996); Stribbling S.M., et al., "Biodistribution of an Antibody-Enzyme Conjugate for Antibody-Directed Enzyme Prodrug Therapy in Nude Mice Bearing a Human Colon Adenocarcinoma Xenograft," *Cancer Chemother Pharmacol,* 40(4):277-84 (1997); Bagshawe K.D., et al., "Developments with Targeted Enzymes in Cancer Therapy," *Curr Opin Immunol,* 11(5):579-83 (1999); Sharma S.K., et al., "Human Immune Response to Monoclonal Antibody-Enzyme Conjugates in ADEPT Pilot Clinical Trial," *Cell Biophys,* 21(1-3):109-20 (1992); Dowell R.I., et al., "New Mustard Prodrugs for Antibody-Directed Enzyme Prodrug Therapy: Alternatives to the Amide Link," *J Med Chem,* 39(5):1100-5 (1996); Connors T.A.; Knox R.J., "Prodrugs in Cancer Chemotherapy," *Stem Cells (Dayt),* 13(5):501-1 (1995); Springer C.J., et al., "Prodrugs of Thymidylate Synthase Inhibitors: Potential for Antibody Directed Enzyme Prodrug Therapy (ADEPT)," *Anti-cancer Drug Des,* 11(8):625-36 (1996); Wallace P.M.; Senter P.D., "Selective Activation of Anti-cancer Prodrugs by Monoclonal Antibody- Enzyme Conjugates," *Methods Find Exp Clin Pharmacol,* 16(7):505-12 (1994); Denny W.A.; Wilson W.R., "The Design of Selectively-Activated Anti-Cancer Prodrugs for Use in Antibody-Directed And Gene-Directed Enzyme-Prodrug Therapies," *J Pharm Pharmacol,* 50(4):387-94 (1998); Senter P.D.; Svensson H.P., "A Summary of Monoclonal Antibody-Enzyme/Prodrug," *Adv Drug Delivery Rev,* 22:341-349 (1996); Roger G. Melton, "Preparation and Purification of Antibody-Enzyme Conjugates for Therapeutic Applications," *Adv Drug Delivery Rev,* 22:289-301 (1996); Roger F. Sherwood, "Advanced Drug Delivery Reviews:

Enzyme Prodrug Therapy," *Adv Drug Delivery Rev,* 22:269-288 (1996); Niculescu-Duvaz I.; Springer C.J., "Antibody-Directed Enzyme Prodrug Therapy (ADEPT): A Review," *Adv Drug Delivery Rev,* 26:151-172 (1997); Ravi V.J. Chari, "Targeted Delivery of Chemotherapeutics: Tumor-Activated Prodrug Therapy," *Adv Drug Delivery Rev,* 31:89-104 (1998); 4,975,278, 12/04/90, Senter, et al., "Antibody-enzyme Conjugates in Combination with Prodrugs for the Delivery of Cytotoxic Agents to Tumor Cells", the contents of which are incorporated herein by reference in their entirety.

Drugs embodied by the present invention can preferably be used at extremely low concentrations in vivo, generally in the nanomolar to picomolar range or lower. At these concentrations the fate of the drug can be defined by high affinity targeting interactions under perhaps nonequilibrium conditions. Typically metabolic enzymes function by forming an enzyme substrate complex that is transformed into the products. In general, the Km for enzymes is in the micromolar range. Accordingly, drug metabolism can predominantly occur at the sites where the drug is trapped by the high affinity binding, provided that the drug has a sufficiently long half-life to allow distribution to the target site. If the drug E-T is selectively localized to the tumor surface and the triggering enzyme is also selectively localized to the tumor surface then greatly enhanced antitumor selectivity can result.

If the free toxin is poorly internalized by cells, then the extracellular liberation of the toxin from E-T can functionally detoxify the drug. In a preferred embodiment, of the present invention applied to this circumstance the trigger can be activated

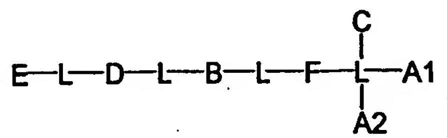
by an enzyme which is enriched in non-tumor cells where dose limiting toxicity takes place. In an even more preferred embodiment of the invention the (detoxifying) trigger can be activated by an enzyme that is selectively delivered to non-tumor cells. For example, the detoxifying trigger can be activated by an enzyme that is coupled to an antibody selective for bone marrow stem cells. This can allow for the selective detoxification of the drug by bone marrow stem cells. Currently, the sparing of bone marrow stem cell toxicity is accomplished by the use of bone marrow transplantation, which is a risky and costly one time procedure. There is a very significant practical advantage to employing a prodrug of the present class along with a detoxifying enzyme that is selectively targeted to vital normal cells. Targeting of normal cells is an easier proposition than targeting tumor cells. The blood supply is generally superior in normal tissues. Most importantly, to achieve a protective effect it can be sufficient to deliver antibody-enzyme conjugate to a minority of the normal bone marrow stem cells. In contrast, to achieve a therapeutic effect (3 log reduction in tumor burden) by targeting the tumor cells it is necessay to deliver antibody enzyme complex to 99.9% of the tumor cells.

The scope of the present invention includes a method of sparing vital normal cells of drug toxicity by targeting, to the normal cells, an enzyme that activates a detoxification trigger on the administered targeted drug that detoxifies the drug.

The scope of the present invention includes the set of a targeted drug with a detoxfication trigger and a targeted enzyme that can activate the detoxification trigger and detoxify or markedly lower the toxicity of the drug.

The scope of the present invention includes a drug that has a detoxification trigger that when activated functionally detoxifies or lowers the toxicity of the drug by interfering with cellular uptake.

In another preferred embodiment of the present invention, E-T comprises the following structure:



wherein A1 and A2 are targeting ligands; B is a trigger that upon activation liberates the effector agent portion of the molecule from the targeting ligands; C is a masked intracellular transport ligand; D is an intracellular trapping ligand or masked intracellular trapping ligand; E is an effector agent; and F is a detoxification trigger that when activated decreases the toxicity or effector activity by interfering with cellular uptake of the effector agent into the cell.

The drug can bind with very high affinity to targeted tumor cells via receptors a1 and a2. At the tumor cells surface either spontaneous chemical processes or enyzymatic processes can trigger the unmasking of the intracellular transporter ligand that is comprised of a ligand that binds to a cellular receptor that then actively transports the complex into the cell. Trigger B can either be activated by intracellular enzymes or be activated extracellularly with a delay mechanism that allows sufficient time for the complex to be transported into the cell prior to the release of the toxin. The trigger, which unmasks the intracellular transporter

ligand, can be activated by enzymes that are enriched in the tumor microenvironment, or by ubiquitous enzymes, or by spontaneous chemical processes. If the unmasking trigger can be activated by a ubiquitous enzyme such as esterase, then it is desirable to incorporate a time delay mechanism. The time delay mechanism can serve to allow time for the targeting receptors rather then the intracellular transport functionality to define the specificity of drug distribution. A time delay mechanism can be made having a triggering event such as the enzymatic cleavage of an ester that initiates a second chemical reaction that proceeds at a rate with the desired half-life. Triggers are described in detail in a latter section.

In one embodiment of the invention, the detoxifying trigger can be activated by an enzyme that is selectively delivered to non-tumor cells. Complementary to this, is this case in which the trigger that unmasks the transport ligand can be activated by an enzyme that is selectively and independently targeted to the tumor cells.

Multifunctional drug delivery vehicles with both toxifying and detoxifying triggers can have the ability to be either toxic or nontoxic to cells depending upon relative rates of activation of the respective trigger functionalities. The drugs have a logic circuit with decision-making ability. The input corresponds to the levels of enzyme activity available to activate the toxifying and detoxifying triggers respectively. The output is increased or decreased drug toxicity for the potential target cell. Glazier previously disclosed a class of anti-cancer drugs that have toxification and detoxification functionalities.

The following reference relates to this subject matter: 5,274,162, 12/28/93, Glazier, "Antineoplastic Drugs with Bipolar Toxification/Detoxification Functionalities."; 5,659,061, 8/19/97, Glazier, "Tumor Protease Activated Prodrugs of Phosphoramide Mustard Analogs with Toxification and Detoxification Functionalities", the contents of which are incorporated herein by reference in their entirety. However, the previously disclosed Antineoplastic Drugs with Bipolar Toxification/Detoxification Functionalities lacked targeting ligands, would need to be used at relatively high doses, and could potentially undergo substantial non-target site metabolism. The present invention can allow for very high affinty multifactorial drug targeting. In preferred embodiments the present drugs can be employed at ultra-low doses under conditions in which drug metabolism (activation of triggers) can be defined by the tumor microenvironment.

The scope of the present invention includes, the class of drugs E-T, wherein the drug binds to the target cell and exerts the biological effector activity of E depending upon the input received by triggers that turn on (or increase) or turn off (or decrease) the biological effector activity of E. This class of drugs enables multifactorial targeting in which the factors or properties that define targeting selectivity and biological activity include both the targeting receptors and triggering factors tr1...trn. The designation "trn" is used to refer to enzymes or biomolecules or other factors that activate a particular trigger referred to as "trigger N" or "TRN."

The scope of the present invention also encompasses the method comprising the following steps:

1.) The administration of one or more targeted drugs E-T that has one or more triggers TR1...TRN that when activated by the triggering factors tr1...trn undergoes either an increase or decrease in drug effector activity.

2.) The administration of one or more compounds (Txn-trn) comprised of targeting groups (Txn) linked to a triggering factor (trn), such that the targeting group delivers the triggering factors to selected population of cells (Pxn); and thereby modulates the biological activity of the drug(s) ET at the population of cells Pxn.

This technology can allow an enhancement of tumor selectivity. Vital normal cell populations can be targeted with triggering factors trn that activate the detoxification trigger and decrease the toxicity of the drug E-T. While tumor cells can be targeted with triggering factors that activate toxifying triggers and thereby enhance the toxicity of the drug E-T.

The triggering factor trn can be a wide range of enzymes that utilize a component of the trigger as a substrate and thereby activate the trigger functionality. The targeting group Txn can be any group or set of groups linked together that bind to the desired population of cells Pxn. Depending upon the context, the targeting group Txn can be selective for tumor cells or for normal cells. A large number of targeting groups selective for tumor cells are described in other sections. Suitable targeting groups include ligands that bind to receptors

that are enriched on tumor cells, monoclonal antibodies, monoclonal antibody analogs, Fab portions or an antibody or monoclonal antibody, growth factor or any other structure which binds selectively to the target cell.

When targeting Txn-tm to normal cells the Txn can be selected to bind to receptors that are enriched on vital normal cells relative to tumor cells. For example, Txn can be a monoclonal antibody specific for the CD34 antigen, which is present on the surface of vital bone marrow stem cells but absent from most tumors. The complex Txn-tm could then be used to selectively detoxify the drug E-T on CD34 + bone marrow stem cells. The following reference relates to this subject matter: Civin CI, et al., "Highly Purified CD34-Positive Cells Reconstitute Hematopoiesis," *J Clin Oncol,* 14(8):2224-33 (1996), the contents of which are incorporated herein by reference in their entirety.

Many malignancies are characterized by the loss of critical membrane proteins. The present method allows the *loss* of one or more of these proteins from tumor cells to be a factor in defining the domain of tumor targeting. In a preferred embodiment, Txn-tm is selected such that Txn binds to a protein or factor that is lost or under-expressed on the surface of tumor cells and tm is comprised of an enzyme that activates a detoxification trigger on the drug E-T. In preferred embodiments, Txn is a monoclonal antibody or monoclonal antibody analog which binds to one of the following membrane associated proteins which is under-expressed in various human cancers: E-cadherin; Transforming growth factor beta receptors; Syndecan-1; Galectin –3; Deleted in colorectal cancer (DCC); Epil or Epitheal Protein Lost in Neoplasm; KAI1 protein; Connexin 43; H-

cadherin; CD38; VLA-2 collagen receptor; P-cadherin; Luminal epithelial antigen (LEA135); Maspin; Mel-Cam; Billiary glycoprotein; Epithelial cell adhesion molecule C-CAM; Beta 4 integrin subunit; and Hemidesmosomal proteins.

Masked Intracellular Transport Ligands

Intracellular delivery is essential for the activity of many drugs. A general method to deliver drugs into cells is to couple the drugs to a ligand such as folic acid, which is taken up by cells via receptor mediated endocytosis. The following reference relates to this subject matter: 5,688,488, 11/18/97, Low, et al., "Composition and Method for Tumor Imaging."; 5,416,016, 5/16/95, Low, et al., "Method for Enhancing Transmembrane Transport of Exogenous Molecules.", the contents of which are incorporated herein by reference in their entirety.

However, the use of an intracellular transport ligand such as folic acid can often define targeting selectivity to the benefit or the detriment of the therapy. If the intracellular transport ligand were simply folic acid then the spectrum of drug distribution and targeting would be significantly defined by the distribution of folate receptors in the body. Folate targeted moieties end up largely in the kidney, which is often undesirable. The following reference relates to this subject matter: Wang S., et al., "Design and Synthesis of [111In]DTPA-Folate for use as a Tumor-Targeted Radiopharmaceutical," *Bioconjug Chem*, 8(5):673-9 (1997), the contents of which is incorporated herein by reference in its entirety.

The properties of a complex of the protein pro-urokinase and saporin serves to illustrate how targeting and internalization can be mechanistically distinct. This complex binds to the urokinase receptor of tumor cells and is internalized

following binding of the saporin to the low-density lipoprotein transport receptor. This example does not involve a masked intracellular transport ligand. The following reference relates to this subject matter: Ippoliti R., et al., "Endocytosis of a Chimera between Human Pro-Urokinase and the Plant Toxin Saporin: An Unusual Internalization Mechanism," *FASEB,* 14(10):1335-1344 (2000), the contents of which is incorporated herein by reference in its entirety.

A compound ET, further comprising a masked intracellular transporter ligand provides a general solution to the problem of efficient intracellular drug transport while retaining targeting selectivity due to the targeting ligands. A masked intracellular transporter ligand is comprised of a group which when unmasked is able to bind to cellular receptors that transport bound ligands into the cell. The current invention allows targeting to be defined by the targeting ligands. A second major advantage is that the cell associated target receptors that provide targeting specificity need not possess the property of being able to transport the targeted drug into the cells. Finally, as discussed below, the masked intracellular transported ligand provides a means by which to provide a simultaneous plurality of intracellular transport mechanisms that can decrease the development of drug resistance.

A variety of masked transporter ligands can be employed. Preferably the following factors are considered individually or in combination in selecting the masked ligand:

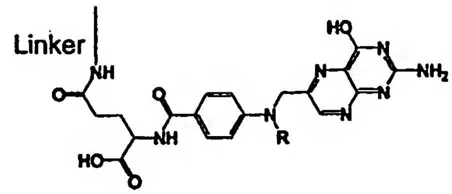
1.) When unmasked the group can bind with sufficient affinity to a structure on the target cell, which can activate transport into the cell;

2.) The group has a chemical moiety which can be modified in a reversible manner such that the modification impairs the ability of the group to bind productively to the cellular transport mechanism (ie., a group that allows for masking);

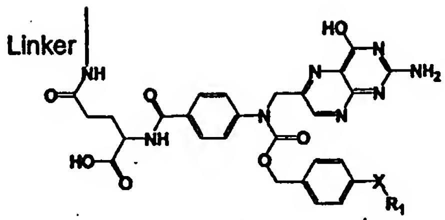
3.) The masked transporter group can be capable of being unmasked by interaction with an enzyme, metabolite, or by a spontaneous chemical process; and

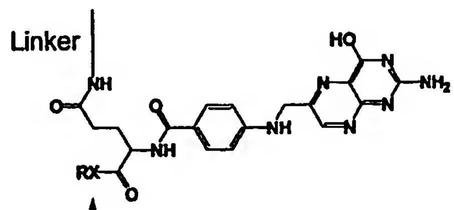
4.) The unmasked intracellular transporter group can bind to a protein or other factor that also binds to a cell membrane receptor and activates intracellular transport of bound ligands.

In a preferred embodiment, the masked intracellular transporter ligand is a folic acid derivative coupled via one of its carboxylate groups, preferably the gamma carboxylate group, through a linker to the rest of the drug, wherein the folic acid is substituted in a bioreversible manner such that binding of the derivative to the folate receptors is impaired in a bioreversible manner. Preferred sites of derivatization are nitrogen 10 or at the alpha carboxy group. A preferred embodiment comprises substitution at the N10 position of the folic acid by a bioreversible amino protecting group referred to as a "trigger" that can be modified in vivo and which, upon this modification referred to as "trigger activation", unmasks the amino group. Another preferred embodiment comprises folic acid substituted at the alpha carboxy group to yield an ester or amide. These are illustrated below:



Masked Folate





Masked Folate

Trigger

Trigger

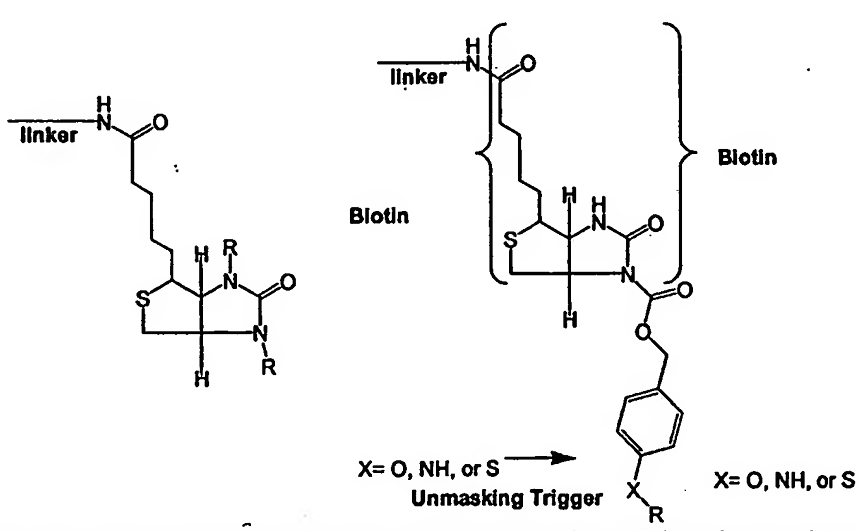
Masked Folate

X= O, NH, or S

wherein R is a trigger group and R1 is a bioreversible protecting group for –X-H, and wherein X is O, NH, or S. Preferred triggers and preferred embodiments of R1 are described in the trigger section of this document. Cleavage of the trigger can unmask the folate and initiate the process of active cell uptake. A wide variety of triggers can be employed including esters, phosphoesters, phosphodiesters, amides, substituted disulfides, oligopeptides, and glycosides. In principle, any functionality suited for use in the ADEPT approach as a trigger could be employed along with an appropriately selected target enzyme that cleaves that trigger. The trigger can be activated by tumor-selective proteases. A description of triggers of this type can be found in: 5,659,061, 8/19/97, Glazier A., "Tumor Protease Activated Prodrugs of Phosphoramide Mustard Analogs with Toxification and Detoxification Functionalities", the contents of which is incorporated herein by reference in its entirety.

In a preferred embodiment, a clock-like time delay trigger is employed to unmask the intracellular transport ligand. Triggers of this type can allow the drug to have time to bind to the tumor prior to unmasking of the intracellular transport ligand. A variety of clock-like time delay triggers are described in the trigger section of the present invention.

In a preferred embodiment of the present invention the masked intracellular transporter ligand comprises biotin that is chemically modified in such a manner as to interfere with receptor binding in a bioreversible manner. Biotin can be linked to the remainder of the drug via its carboxylate group and can retain binding affinity to biotin receptors. A preferred embodiment comprises biotin with bioreversible substitution of one or more of the ureido amidic protons as illustrated below:



X and R can be groups as described previously for the masked folate trigger. Drugs of this class (with a masked biotin receptor) can be administered in conjunction with one or more transporter moieties to which is coupled a biotin

binding factor such as avidin or streptavidin. The transporter moieties are selected such, they bind to receptors on the tumor cell surface and are internalized. Binding of the unmasked biotin to the administered avidin-transporter moiety can transport the drug complex into the tumor cell. The avidin-transporter moiety can be tumor-selective or non-selective without specificity for tumor cells. Its role is to efficiently deliver the drug already targeted and located on the tumor cell surface into the cell. It is preferred to administer the drug first, allow time for the tumor localization to occur and then to administer the avidin-transporter moiety. Although it can be pointed out that high affinity between the drug and the avidin-transporter can only occur after the biotin is unmasked. The avidin-transporter can be given intravenously at a sufficiently high dose to allow contact with the tumor cells. It is preferable to use simultaneously at least two different types of avidin-transporters to avoid the selection of tumor drug resistance based on lack of binding or impaired internalization of one particular type of avidin-transporter.

Some considerations for the avidin-transporter moiety are as follows:

1.) Avidin can be coupled to the transporter function in a fashion that does not impede high affinity biotin binding;

2.) The transporter function can bind to the target cells and be internalized; and

3.) The avidin-transporter can be of low toxicity.

Any protein, hormone, lipid, nutrient, or substance, which is internalized by cells by efficient endocytotic proceess, that can be coupled to a biotin-binding moiety such as avidin can be employed. Preferred transporter moieties include: transferrin, alpha 2 macroglobulin, insulin, folic acid, and epidermal growth

factor. Monoclonal antibodies against receptors or occupied receptor complexes known to undergo endocytosis can also be used. Techniques for coupling biotin-binding factors such as avidin to other moieties are well known. The following reference relates to this subject matter: Mukherjee S., et al., "Endocytosis", *Physiological Reviews*,77(3):759-803 (1997); Hanover John A.; Dickson Robert B. (1985) Transferrin: Receptor-Mediated Endocytosis and Iron Delivery. *in* "Endocytosis" (I. Pastan and M. Caningham, eds.), pp.131-161. Plenum Press, New York; Hermanson Greg T. (1996) "Bioconjugate Techniques." Academic Press, Inc., the contents of which are incorporated herein by reference in their entirety.

The scope of the present invention includes compounds comprised of one or more masked intracellular transport ligands and the method of delivering drugs or other effector molecules into cells by contacting the cells with a compound that has one or more masked intracellular transport ligands.

The scope of the present invention also includes the method of delivering drugs and effector molecules into cells that comprises contacting the cells with a targeted drug ET and also contacting the cells with one or more targeted transport moiety that facilitates drug transport into the cell. In a preferred embodiment the drug ET and targeted transport moiety are each targeted to different targets present on the target cells. In a preferred embodiment the target is a tumor cell. Preferred tumor-selective targets are described throughout this document.

The scope of the present invention includes a preferred embodiment that comprises a method of delivering a targeted drug or effector molecule into cells by multiple independent non-target endocytotic receptors. This method can be useful to circumvent drug resistance due to the loss of a single intracellular transport or endocytotic receptor.

Another preferred embodiment of the present invention comprises the following structure:

$$\begin{array}{ccccccccc} & & & & & & E2 & & \\ & & & & & & | & & \\ & & & & & & B2 & & \\ & & & & & & | & & \\ E1 & — & L & — & B1 & — & L & — & A1 \\ & & & & & & | & & \\ & & & & & & A_2 & & \end{array}$$

wherein A1 and A2 are targeting ligands; B1 and B2 are triggers that upon activation liberate the effector agent portion of the molecule from the targeting ligands and E is an effector agent. This embodiment incorporates, in addition to the features discussed previously, an effector mechanism comprised of two different cytotoxic agents, which can be released by two different triggering mechanisms. This feature can markedly decrease the rate at which tumor resistance develops to the drugs without significantly increasing overall drug toxicity. In addition, this can allow the joint delivery of two drugs that exhibit synergistic toxicity. In the preferred embodiment, the toxins are selected such that resistance to each is mediated by independent mechanisms. For example, if tumor resistance to one of the toxins is mediated by MDR1 gene product then ideally the second toxin can retain activity in cells expressing this phenotype. The following reference relates to this subject matter: Gottesman Michael M., "How Cancer Cells Evade Chemotherapy" Sixteenth Richard and Hinda

Rosenthal Foundation Award Lecture", *Cancer Research*, 53:747-754 (1993), the contents of which is incorporated herein by reference in its entirety.

A preferred embodiment of the present invention is the anti-cancer compound ET comprised both of a cytotoxic moiety(s) and an inhibitor to multi-drug resistance mechanisms such as MDR1 P- glycoprotein. This can allow major mechanisms of tumor drug resistance to be overcome at a target specific level without increasing total systemic toxicity. The emergence of tumor resistance to a broad range of unrelated antineoplastic drugs by increased expression of the multi-drug transporter P-glycoprotein, which actively transports the drugs out of the tumor cells, is a major and fundamental limitation in cancer treatment. There have been extensive efforts towards the development of inhibitors to MDR P-glycoprotein. Clinical trials to date have been unsuccessful and complicated by systemic toxicity. The present invention can allow for the selective delivery of the multi-drug resistance inhibitors to tumor cells concurrently with the selective delivery of the anti-cancer drugs.

Targeting Specificity

The present invention can be used to target drugs to essentially any type of cell, cell population, tissue, or tissue type. The targeting specificity or targeting domain of multifunctional drug delivery vehicles can be defined as the populations of cells that are subjected to the effector action of the drug. The targeting domain of multifunctional drug delivery vehicles is a multifactorial or multivariable function in which the variables are targeting ligands specificity,

specificity of triggers, and nature of the effector agent ultimately delivered. It is the interaction between these variables that ultimately defines the targeting domain and can allow exquisitely specific tumor targeting despite the fact that no single factor is unique to tumors.

The initial targeting specificity of the drugs can be defined by the combined high affinity interactions of the drug targeting ligands A1,...An with the target cell associated receptors a1,...an. The drugs are to be administered at a dose sufficient to bind an effective quantity to the targeted cell population or at a dose sufficient to evoke the desired therapeutic activity. For some of the preferred embodiments of the present invention the concentration range can generally be in the nanamolar to picomolar range or lower. The use of excessive concentrations can allow secondary non-targeting factors to dominate the pattern of drug distribution and metabolism with a potential reduction in the targeting selectivity and therapeutic index. The present class of drugs can be orders of magnitude more potent for targeted cells than the non-target toxin due to the high receptor mediated affinity of the drug to the targeted cells.

The targeting ligands A1,...An can be selected to bind a large variety of receptors a1,...an which are present at increased amounts on the surface of tumor cells compared to vital normal cells. The terms "target selective" and "tumor-selective" are used in a functional sense in this patent application. Absolute selectivity is elusive. Drugs always have some form of dose limiting toxicity that restricts the therapeutic index. A target can be considered tumor-selective if it is enriched on tumor cells compared to vital normal cells in the

tissue that ordinarily suffers dose limiting toxicity. For example, if the dose limiting toxicity of the parent drug being delivered is bone marrow toxicity then a receptor or enzyme enriched on tumor cells compared to bone marrow stem cells would be a suitable "tumor-selective" target even if this target is not unique to tumor cells. Normal enzymes or receptors in abnormal locations can also function as tumor-selective targets and are a biochemical manifestation of metastasis. For example, if an enzyme is ordinarily confined to the luminal surface of the gastrointestinal tract the presence of that enzyme on malignant cells metastatic to the liver can be used for selective targeting. This can be accomplished by employing a drug that is given intravenously and fails to penetrate to the luminal surface of the GI tract. (Alternatively, the target sites on the normal GI cells can be blocked by an orally nonabsorbable inhibitor to the receptor or enzyme.) Useful tumor-selective targets can also be receptors or enzymes that are present on both malignant cells and normal cells provided that the targeted normal cells are not vital for life. Normal enzymes that are present intracellularly in normal cells but released or activated extracellularly in the tumor microenvironment can also be used for selective targeting provided that the drug is designed to remain in the extracellular space.

The targeted cell receptors can be any chemical moiety that is enriched on the target cells relative to the cell populations which one desires not to target. With the advent of combinatorial chemistry, and high throughput automated screening it is now possible to select high affinity ligands that can bind to essentially any biological receptor. The following reference relates to this subject matter: Wilson, Stephen R.; Czarnik, Anthony W.(eds.), "Combinatorial Chemistry;

Synthesis and Application." John Wiley & Sons, Inc., the contents of which is incorporated herein by reference in its entirety.

The steps in this process are well known to one skilled in the arts and include:

1.) Coupling a large library of potential receptor binding ligands to a linker and reporter functionality such as a fluorescent group, an enzyme, or a group such as biotin which can be readily detected;

2.) Coupling the receptor moiety to a solid phase;

3.) Incubating the receptor ligand-detector molecules with the receptor;

4.) Washing to remove unbound ligand; and

5.) Assaying for the reporter functionality bound to the receptor to identify high afffinity binding ligands.

For example, one can couple a fluorescent derivative via a linker to a library of millions of compounds and screen potential ligands for binding affinity to the desired receptor using a fluorescent based binding assay.

The hallmark of malignancy is uncontrolled cell proliferation and tissue invasion. Neither the processes of cell replication nor the enzymology of tissue invasion (remodeling) are by themselves uniquely diagnostic of malignancy. But jointly, these processes likely can provide highly selective criteria to define effective targeting for the treatment of malignancy. The current class of multifunctional anti-cancer drugs provides the opportunity to have anti-cancer agents that are targeted simultaneously and jointly to both the proliferative and the invasive character of malignant cells.

Antineoplastic agents directed against cell replication are well-known and typified by anti-cancer drugs such as alkylating agents, topoisomerase inhibitors, DNA antimetabolites, DNA polymerase inhibitors, and antimitotic agents. Targeting such drugs to cells that express the property of tissue invasiveness can significantly increase antitumor selectivity. Since the biochemical expression of tissue invasiveness is an essential component of malignancy, the development of tumor resistance by loss of these properties can be incompatable with persistence of the malignant phenotype. It is precisely for this reason that cytotoxic targeting towards the coupled expression of invasiveness and proliferation is so compelling. It is also important to recognize that tumors are composed of a heterogenous population with the most invasive and malignant cells defining the ultimate clinical outcome.

Many targeting receptors and receptor combinations are unrelated to cell survival or the processes of malignancy. Resistance to drugs directed towards these targeting features is predictable and expected. However, even if only a two log reduction in tumor burden is obtained prior to the development of resistance to the specific targeted drug by loss of the nonessential target receptor sites by the tumor cells, the net result is useful towards the overall goal of achieving sufficient log reductions of tumor burden to completely eliminate the disease.

There are five general classes of receptors which can be employed as "tumor-selective targets":

1.) Enzymes and factors that are related to the biochemical manifestations of uncontrolled cell growth. Some examples include: autocrine growth factors, and abnormal receptor tyrosine kinases;

2.) Enzymes and molecules that are expressed by the tumor cells or in the microenvironment of tumor cells that are involved in the mechanism of tissue invasion. Some examples include collagenases, plasmin, urokinases, metalloproteinases, cathepsins, heparanase;

3.) Normal enzymes and receptors present in abnormal locations in association with tumor cells. Examples include: trypsin in ovarian cancer, sucrase-isomaltase in colon adenocarcinoma, pepsin in breast adenocarcinoma, and dipeptide transporter (PEPT1) colon adenocarcinoma;

4.) Normal enzymes and molecules associated with both tumor cells and normal tissue provided that the normal tissue is not vital to life or not sensitive to the delivered anti-cancer drug. Examples include: prostatic membrane surface antigen, prostatic specific antigen, hepsin in ovarian cancer, and neutral endopeptidase in leukemia; and

5.) Receptors unique to tumor cells, such as tumor specific antigens.

Suitable receptor targets include enzymes that are membrane associated with the target cell or which bind to receptors on the target cell, structural components of the target cell, or hormone receptors on the target cell. It is important to emphasize the point that targets, which individually cannot provide sufficient specificity in combination with the multifunctionality of the present invention, can provide useful targeting selectivity and in preferred cases can

provide excellent target specificity. Targets also can be localized to the microenvironment of tumors. This is discussed in more detail in the section on targeted immunotherapy.

Targeting ligands can also bind to intracellular receptors that are enriched in target cells. For most anti-cancer drugs the biological activity is dependent upon intracellular concentration that is a function of the relative rates of drug influx and drug efflux. Many anti-cancer drugs are actively pumped out of cells by p-glycoprotein and related proteins. This is a major mechanism of tumor resistance to antineoplastic drugs. Intracellular targeting ligands that bind to intracellular receptors that are enriched in target cells can contribute to drug selectivity by trapping drug selectively in target cells. A variety of specific and non-specific intracellular trapping ligands are described elsewhere in this patent.

Preferred embodiments (embodiments TF#.X, wherein X is the number given below) include the anti-cancer compounds ET comprised of targeting ligands, triggers, and effector agents that are selective for combinations of the following factors or targeting properties:

1) 5'nucleotidase
2) 5-aminoimidazole-4-carboxamide ribonucleotide transferase
3) a cathepsin type protease
4) a collagenase
5) a gelatinase
6) a matrix metalloproteinase
7) a membrane type matrix metalloproteinase

8) acid phosphatase

9) activated Factor X

10) adenine phosphoribosyltransferase

11) alkaline phosphatase

12) alpha v beta 3 integrin

13) amino-peptidase N

14) androgen receptor

15) aspartate transcarbamylase

16) basic fibroblast growth factors and their receptors

17) bombesin /gastrin releasing peptide receptors

18) carbamoyl phosphate synthetase

19) carboxypeptidase M

20) cathepsin B

21) cathepsin D

22) cathepsin K

23) cathepsin L

24) cathepsin O

25) CD44

26) CXCR4 receptor

27) deoxycytidine kinase

28) deoxyguanosine kinase

29) dihydrofolate reductase

30) dihydroorotate dehydrogenase

31) dipeptidyl peptidase IV

32) emmprin

33) epidermal growth factor receptors and related proteins

34) epidermal growth factors

35) estrogen receptor

36) Fas ligand

37) fibroblast activation protein

38) folate binding receptors

39) galactosyltransferase

40) gamma-glutamyl transpeptidase

41) gastrin/cholecystokinin type B receptor

42) GDP-L-fucose:beta-D-galactoside alpha-2-L-fucosyltransferase

43) glutamate carboxypeptidase II or Prostate-specific membrane antigen

44) glutathione S –transferase

45) glycinamide ribonucleotide transformylase

46) gonadotropin releasing hormone receptor

47) GPIIb/IIIa fibrinogen receptor

48) guanidinobenzoatase

49) heparanase

50) hepsin

51) human glandular kallikrein 2

52) compounds made reactive or modified in vivo as the result of hypoxia

53) hypoxanthine-guanine phosphoribosyltransferase

54) inosine 5'monophosphate dehydrogenase

55) insulin-like growth factor receptors

56) insulin-like growth factors

57) laminin receptor

58) leutinizing hormone releasing receptor

59) matrilysin

60) matripase

61) melanocyte stimulating hormone receptor

62) mitogen activated protein kinase

63) multi-drug resistance protein

64) nerve growth factors and their receptors

65) neuroleukin/ phosphohexose isomerase /autocrine motility factor

66) neuropeptide Y receptors

67) neutral endopeptidase

68) nitrobenzylthioinosine-binding receptors (nucleoside transporter)

69) norepenephrine transporters

70) nucleoside transporter proteins

71) opioid receptors

72) orotidine-5'-phosphate decarboxylase

73) oxytocin receptor

74) p53 antigen

75) patelet derived growth factor receptor

76) pepsin c

77) peripheral benzodiazepam binding receptors

78) p-glycoprotein

79) phospatidylinositol 3-kinase

80) placental alkaline phosphatase

81) plasmin

82) platelet-derived growth factors and their receptors

83) polyamine transporters

84) porphyrin receptors

85) progesterone receptors

86) prolactin receptor

87) prostate specific antigen

88) prostatic acid phosphatase

89) protein kinase A

90) ribonucleotide diphosphate reductase

91) ribonucleotide reductase

92) seprase

93) sex hormone globulin binding receptor

94) sigma receptors

95) somatostatin receptors

96) SP220K

97) Src kinase

98) steroid sulfatase

99) stromelysin 3

100) sucrase-isomaltase

101) TADG14

102) Thiolesterase II

103) thrombin

104) thrombin receptor

105) thymidine kinase

106) thymidylate synthase

107) tissue factor

108) tissue plasminogen activator

109) TMPRSS2

110) transferrin receptors

111) transforming growth factors and their receptors

112) transporter (PEPT1)

113) trypsin

114) tumor necrosis factor receptor

115) type IV collagenase

116) uridine/cytidine kinase

117) urokinase

118) vacuolar type proton pump (V- ATPase)

119) xanthine-guanine phosphoribosyltransferase

120) any tumor-selective antigen

121) any tissue specific antigen which is present on tumor cells, but absent from vital normal cells

Tumor-selective Targets and Targeting Ligands:

The targeting ligands described below are preferred embodiments of targeting ligands for anti-cancer drugs ET of the present invention and all targeted anti-cancer drugs that are embodiments of the present invention:

Laminin Receptors

The laminin receptor is a membrane associated protein which binds laminin, elastin and, type IV collagen. The receptor facilitates the cell adhesion and migration key components of invasiveness characteristic of malignancy. The laminin receptor is over-expressed in a large number of malignancies including: breast, colon, prostate, ovarian, renal, pancreatic, melanoma, thyroid, lung, lymphomas, leukemias, gastric, and hepatocellular cancer. It is strongly associated with metastatic ability and is an independent adverse prognostic in breast, prostate, lung, thyroid and gastric cancer. The following references relate to this subject matter: Viacava P., et al., "The Spectrum of 67-kD Laminin Receptor Expression in Breast Carcinoma Progression," *J Pathol,* 182:36-44 (1997); Menard S., et al., "Immunodetection of Bone Marrow Micrometastases in Breast Carcinoma Patients and its Correlation with Primary Tumour Prognostic Features," *Br J Cancer,* 69(6):1126-9 (1994); Putz E., et al., "Phenotypic Characteristics of Cell Lines Derived from Disseminated Cancer Cells in Bone Marrow of Patients with Solid Epithelial Tumors: Establishment of Working Models for Human Micrometastases," *Cancer Res,* 59(1):241-8 (1999); Hipfel R., et al., "Specifically Regulated Genes in Malignant Melanoma Tissues Identified by Subtractive Hybridization," *Br J Cancer,* 82(6):1149-57 (2000); Pelosi G., et al., 'High-Affinity Monomeric 67-Kd Laminin Receptors and Prognosis in Pancreatic Endocrine Tumours," *J Pathol,* 183(1):62-9 (1997); Sanjuan X., et al., " Over-expression of the 67-kD Laminin Receptor Correlates with Tumour Progression in Human Colorectal Carcinoma," *J Pathol,* 179(4):376-80 (1996); van den Brule F.A., et al., "Expression of the 67 kD Laminin Receptor in Human Ovarian Carcinomas as Defined by a Monoclonal

Antibody, MLuC5," *Eur J Cancer,* 32A(9):1598-602 (1996); Hand P.H., et al., "Expression of Laminin Receptor in Normal and Carcinomatous Human Tissues as Defined by a Monoclonal Antibody," *Cancer Res,* 45(6):2713-9 (1985); Cioce V., et al., "Increased Expression of the Laminin Receptor in Human Colon Cancer," *J Natl Cancer Inst,* 83:29-36 (1991); Massia S.P., et al., "Covalently Immobilized Laminin Peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) Supports Cell Spreading and Co-Localization of the 67-Kilodalton Laminin Receptor with Alpha-Actinin and Vinculin," *J Biol Chem,* 268(11):8053-9 (1993); Nadji M., et al., "Laminin Receptor in Lymph Node Negative Breast Carcinoma," *Cancer,* 85(2):432-6 (1999); Terranova V.P., et al., "Laminin Receptor on Human Breast Carcinoma Cells," *Proc Natl Acad Sci USA,* 80(2):444-8 (1983).Montuori N., et al., "Laminin Receptors in Differentiated Thyroid Tumors: Restricted Expression of the 67-Kilodalton Laminin Receptor in Follicular Carcinoma Cells," *J Clin Endocrinol Metab,* 84(6):2086-92 (1999); Fontanini G., et al., "67-Kilodalton Laminin Receptor Expression Correlates with worse Prognostic Indicators in Non-Small Cell Lung Carcinomas," *Clin Cancer Res,* 3(2):227-31 (1997); Menard S., et al., "New Insights into the Metastasis-Associated 67 kD Laminin Receptor," *J Cell Biochem,* 6792):155-65 (1997); de Manzoni G., et al., "Prognostic Significance of 67-kDa Laminin Receptor Expression in Advanced Gastric Cancer," *Oncology,* 55(5):456-60 (1998); Wewer U.M., et al., "Role of Laminin Receptor in Tumor Cell Migration," *Cancer Res,* 47(21):5691-8 (1987); Menard S., et al., "The 67 kDa Laminin Receptor as a Prognostic Factor in Human Cancer," *Breast Cancer Res Treat,* 52(1-3):137-45 (1998); Zheng S., et al., "The Relationship between 67KD Laminin Receptor Expression and Metastasis of Hepatocellular Carcinoma," *J Tongji Med Univ,* 17(4):200-2

(1997); van den Brule F.A., et al., "Expression of the 67-kD Laminin Receptor, Galectin-1, and Galectin-3 in Advanced Human Uterine Adenocarcinoma," *Hum Pathol,* 27(11):1185-91 (1996); Waltregny D., et al., "Brief Communication. Independent Prognostic Value of the 67-kD Laminin Receptor in Human Prostate Cancer," *J Natl Cancer Inst,* 89(16):1224-1227 (1997), the contents of which are incorporated herein by reference in their entirety.

The laminin receptor, although highly over-expressed in many malignancies, is a normal cellular component of many tissues especially endothelial cells. The very low levels of laminin receptor in normal bone marrow cells is of significance as bone marrow toxicity is dose limiting for most anti-cancer drugs. The following references relate to this subject matter: Hand P.H., et al., "Expression of Laminin Receptor in Normal and Carcinomatous Human Tissues as Defined by a Monoclonal Antibody," *Cancer Res,* 45(6):2713-9 (1985) ;Hilario E., et al., "Presence of Laminin and 67kDa Laminin-Receptor on Endothelial Surface of Lung Capillaries. An Immunocytochemical Study," *Histol Histopathol,* 11(4):915-8 (1996); Montuori N., et al., "Expression of the 67-kDa Laminin Receptor in Acute Myeloid Leukemia Cells Mediates Adhesion to Laminin and is Frequently Associated with Monocytic Differentiation," *Clin Cancer Res,* 5(6):1465-72 (1999), the contents of which are incorporated herein by reference in their entirety.

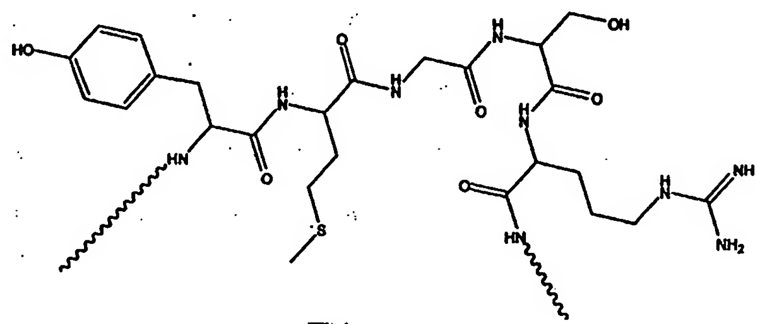
The laminin receptor binds with high affinity to a number of oligopeptides that are related to laminin or elastin. Laminin receptor antagonists have been shown to inhibit metastasis in animals. Radiolabelled laminin binding analogs and

monoclonal antibodies specific for the laminin receptor have been explored as potential diagnostic and or therapeutic agents. The following references relate to this subject matter: Maeda M., et al., "Amino Acids and Peptides. XXXIII. A Bifunctional Poly(Ethylene Glycol) Hybrid of Laminin-Related Peptides," *Biochem Biophys Res Commun,* 248(3):485-9 (1998); Graf J., et al., "A Pentapeptide from the Laminin B1 Chain Mediates Cell Adhesion and Binds the 67,000 Laminin Receptor," *Biochemistry,* 26(22):6896-900 (1987); Rahman A., et al., "Anti-Laminin Receptor Antibody Targeting of Liposomes with Encapsulated Doxorubicin to Human Breast Cancer Cells in Vitro," *J. Natl Cancer Inst,* 81:1794-1800 (1989); Mu Y., et al., "Bioconjugation of Laminin Peptide YIGSR with Poly(Styrene Co-Maleic Acid) Increases its Antimetastatic Effect on Lung Metastasis of B16-BL6 Melanoma Cells," *Biochem Biophys Res Commun,* 255(1):75-9 (1999); Mu Y., et al., "Bioconjugation of Laminin-Related Peptide YIGSR with Polyvinyl Pyrrolidone Increases its Antimetastatic Effect due to a Longer Plasma Half-Life," *Biochem Biophys Res Commun,* 264(3):763-7 (1999); Iwamoto Y., et al., "YIGSR, a Synthetic Laminin Peptide, Inhibits the Enhancement by Cyclophosphamide of Experimental Lung Metastasis of Human Fibrosarcoma Cells," *Clin Exp Metastasis,* 10(3):183-9 (1992); Massia S.P., et al., "Covalently Immobilized Laminin Peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) Supports Cell Spreading and Co-Localization of the 67-Kilodalton Laminin Receptor with Alpha-Actinin and Vinculin," *J Biol Chem,* 268(11):8053-9 (1993); Koliakos G., et al., "Lung Carcinoma Imaging using a Synthetic Laminin Derivative Radioiodinated Peptide YIGSR," *J Nucl Med,* 38(12):1940-4 (1997); Zhao M., et al., "Synthetic Laminin-Like Peptides and Pseudopeptides as Potential Antimetastatic Agents," *J Med Chem,* 37(20):3383-8 (1994); Hinek A.,

et al., "The 67-kD Elastin/Laminin-Binding Protein is Related to an Enzymatically Inactive, Alternatively Spliced Form of Beta-Galactosidase," *J Clin Invest,* 91(3):1198-205 (1993); Iwamoto Y., et al., "YIGSR, a Synthetic Laminin Pentapeptide, Inhibits Experimental Metastasis Formation," *Science,* 238(4830):132-4 (1987); Blood C.H., et al., "Identification of a Tumor Cell Receptor for VGVAPG, an Elastin-Derived Chemotactic Peptide," *J Cell Biol,* 107(5):1987-93 (1988); Grosso L.E.; Scott M., "PGAIPG, a Repeated Hexapeptide of Bovine and Human Tropoelastin, is Chemotactic for Neutrophils and Lewis Lung Carcinoma Cells," *Arch Biochem Biophys,* 305(2):401-4 (1993); Grosso L.E.; Scott M., "Peptide Sequences Selected by BA4, a Tropoelastin-Specific Monoclonal Antibody, are Ligands for the 67-Kilodalton Bovine Elastin Receptor," *Biochemistry,* 32(48):13369-74 (1993); Mecham R.P., et al., "Elastin Binds to a Multifunctional 67-Kilodalton Peripheral Membrane Protein," *Biochemistry,* 28(9):3716-22 (1989); Mecham R.P., et al., "The Elastin Receptor Shows Structural and Functional Similarities to the 67-kDa Tumor Cell Laminin Receptor," *J Biol Chem,* 264(28):16652-7 (1989); 5,567,408, 10/22/96, Zamora, "YIGSR Peptide Radiopharmaceutical Applications"; 5,556,609, 9/17/96, Zamora, "YIGSR Peptide Radiopharmaceutical Applications"; 5,759,515, 6/02/98, Rhodes, et al., "Polyvalent Peptide Pharmaceutical Applications"; 5,231,082, 7/27/93, Schasteen, "Cyclic Peptide with Anti-Metastasis Activity"; 5,092,885, 3/03/92, Yamada, et al., "Peptides with Laminin Activity"; 5,039,662, 8/13/91, Schasteen, "Peptide with Anti-Metastasis Activity"; 4,565,789, 1/21/86, Liotta, et al., "Cell Matrix Receptor System and Use in Cancer Diagnosis and Management", the contents of which are incorporated herein by reference in their entirety.

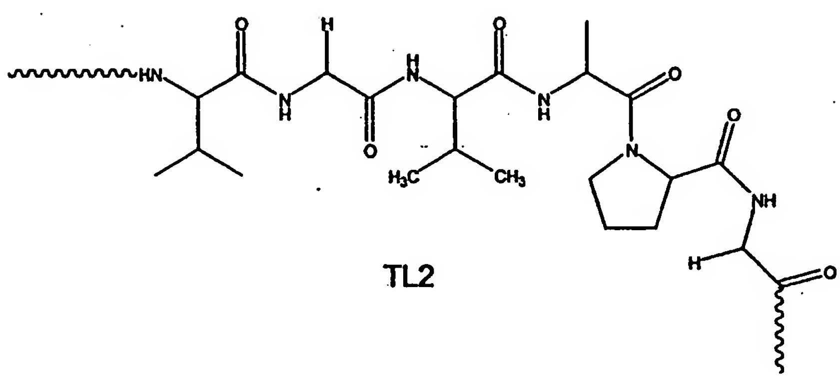
A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to a laminin receptor binding ligand.

In preferred embodiments (embodiments TL1, TL2, TL3, TL4, and TL5), the targeting ligand comprises the following structures:



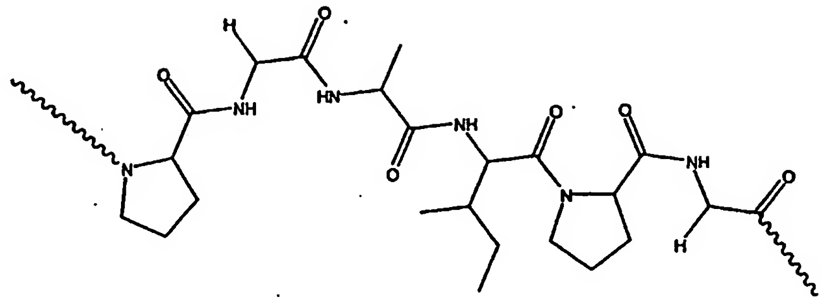
TL1

Or

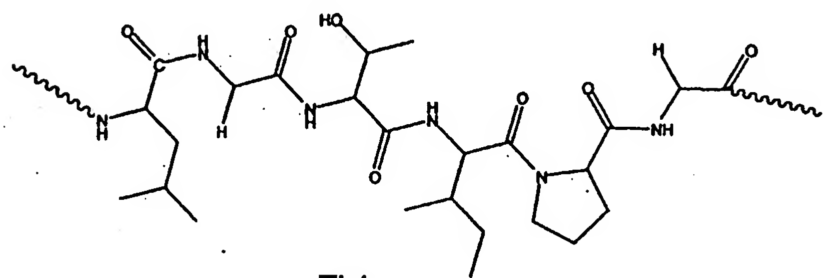


TL2

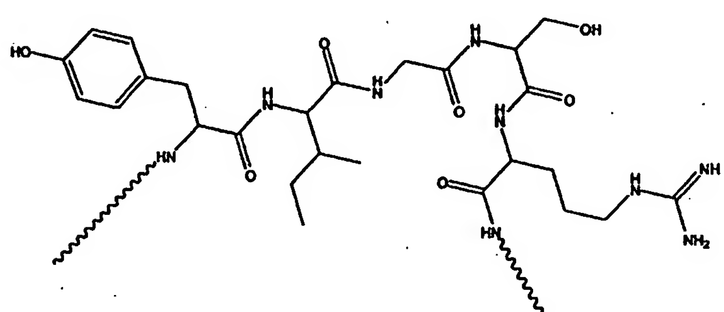
or



TL3



TL4



TL5

wherein the wavy line is H, OH, NH_2, or the site of linker attachment to the remainder of the drug complex; and wherein the amino acid residues have the L-configuration, or the D configuration, or are a racemic mixture.

Integrin alpha V beta 3

Integrin alpha V beta 3 ($\alpha_V\beta_3$) are cell adhesion molecules which bind to RGD peptide sequences present in many extracellular matrix proteins. $\alpha_V\beta_3$ is over-expressed on tumor cells in a number of important malignancies including: melanoma, breast cancer metastatic to bone, ovarian cancer, and neuroblastoma. In addition, $\alpha_V\beta_3$ over-expressed by endothelial cells in tumor neovasculature. $\alpha_V\beta_3$ expression is a strong adverse prognostic indicator in patients with breast cancer. $\alpha_V\beta_3$ is not unique to tumors or tumor neovasculature and is also expressed by platlets, osteoclasts, endothelial cells during wound repair, and by vascular smooth muscle cells. Antagonists and monoclonal antibodies to $\alpha_V\beta_3$ inhibit angiogenesis and tumor growth. Radiolabelled ligands for $\alpha_V\beta_3$ have been described as potential tumor imaging agents. Doxorubicin conjugates of integrin ligands have been described as potential anti-cancer drugs. Monoclonal antibodies to $\alpha_V\beta_3$ are used to reduce coronary artery restenosis following angioplasty. The following references relate to this subject matter: Horton M.A., et al., "The Alpha V Beta 3 Integrin 'Vitronectin Receptor'," *Int J Biochem Cell Biol,* 29(5):721-5 (1997); Pasqualini R, et al., "Alpha V Integrins as Receptors for Tumor Targeting by Circulating Ligands," *Nat Biotechnol,* 15(6):542-6 (1997); Luna J., et al., "Antagonists of Integrin Alpha v Beta 3 Inhibit Retinal Neovascularization in a Murine Model," *Lab Invest,* 75(4):563-73 (1996); Brooks P.C., et al., "Antiintegrin Alpha V Beta 3 Blocks Human Breast Cancer Growth and Angiogenesis in Human Skin," *J Clin Invest,* 96(4):1815-22 (1995); Rabb H., et al., "Alpha-V/beta-3 and alpha-V/beta-5 Integrin Distribution in Neoplastic Kidney," *Am J Nephrol,* 16(5):402-8 (1996).Timar J., et al., "Expression and Function of the High Affinity AlphaII/beta3 Integrin in Murine Melanoma Cells," *Clin Exp Metastasis,*

16(5):437-45 (1998); Deryugina E.I., et al., "Functional Activation of Integrin Alpha V Beta 3 in Tumor Cells Expressing Membrane-Type 1 Matrix Metalloproteinase," *Int J Cancer,* 86(1):15-23 (2000); Gladson C.L., et al., "Expression of Integrin Alpha v Beta 3 in Small Blood Vessels of Glioblastoma Tumors," *J Neuropathol Exp Neurol,* 55(11):1143-9 (1996); Platten M., et al., "Transforming Growth Factors Beta(1) (TGF-beta(1)) and TGF-beta(2) Promote Glioma Cell Migration via Up-Regulation of Alpha(V)Beta(3) Integrin Expression," *Biochem Biophys Res Commun,* 268(2):607-11 (2000); Trusolino L., et al., "Growth Factor-Dependent Activation of Alphavbeta3 Integrin in Normal Epithelial Cells: Implications for Tumor Invasion," *J Cell Biol,* 142(4):1145-56 (1998); Max R., et al., "Immunohistochemical Analysis of Integrin Alpha Vbeta3 Expression on Tumor-Associated Vessels of Human Carcinomas," *Int J Cancer,* 71(3):320-4 (1997); Racanelli A.L., et al., "Inhibition of Neointima Formation by a Nonpeptide Alpha(V)Beta(3) Integrin Receptor Antagonist in a Rabbit Cuff Model," *J Cell Biochem,* 77(2):213-20 (2000); Liapis H., et al., "Integrin Alpha V Beta 3 Expression by Bone-Residing Breast Cancer Metastases," *Diagn Mol Pathol,* 5(2):127-35 (1996); Brassard D.L., et al., " Integrin Alpha(V)Beta(3)-Mediated Activation of Apoptosis," *Exp Cell Res,* 251(1):33-45 (1999); Brooks P.C., et al., "Localization of Matrix Metalloproteinase MMP-2 to the Surface of Invasive Cells by Interaction with Integrin Alpha V Beta 3," *Cell,* 85(5):683-93 (1996); DeNardo S.J., et al., "Neovascular Targeting with Cyclic RGD Peptide (Crgdf-ACHA) to Enhance Delivery of Radioimmunotherapy," *Cancer Biother Radiopharm,* 15(1):71-9 (2000); Kerr J.S., et al., "Novel Small Molecule Alpha V Integrin Antagonists: Comparative Anti-Cancer Efficacy with Known Angiogenesis Inhibitors," *Anti-*

cancer, 19(2A):959-68 (1999); Liapis H., et al., "Expression of Alpha(V)Beta3 Integrin is Less Frequent in Ovarian Epithelial Tumors of Low Malignant Potential in Contrast to Ovarian Carcinomas," *Hum Pathol,* 28(4):443-9 (1997); Gasparini G., et al., " Vascular Integrin Alpha(V)Beta3: A New Prognostic Indicator in Breast Cancer," *Clin Cancer Res,* 4(11):2625-34 (1998); Lanza P., et al., "Selective Interaction of a Conformationally-Constrained Arg-Gly-Asp (RGD) Motif with the Integrin Receptor Alphavbeta3 Expressed on Human Tumor Cells," *Blood Cells Mol Dis,* 23(2):230-41 (1997); Romanov V.I. et al., "RGD-Recognizing Integrins Mediate Interactions of Human Prostate Carcinoma Cells with Endothelial Cells in Vitro," *Prostate,* 39(2):108-18 (1999); Trikha M., et al., "Role of AlphaII(B)Beta3 Integrin in Prostate Cancer Metastasis," *Prostate,* 35(3):185-92 (1998); Cheresh D.A., "Structure, Function and Biological Properties of Integrin Alpha V Beta 3 on Human Melanoma Cells," *Cancer Metastasis Rev,* 10(1):3-10 (1991); Shahan T.A., et al., "Regulation of Tumor Cell Chemotaxis by Type IV Collagen is Mediated by a Ca(2+)-Dependent Mechanism Requiring CD47 and the Integrin Alpha(V)Beta(3)," *J Biol Chem,* 275(7):4796-802 (2000); Singh B., et al., "Vascular Expression of the Alpha(V)Beta(3)-Integrin in Lung and Other Organs," *Am J Physiol Lung Cell Mol Physiol,* 278(1):L217-26 (2000); Clark R.A., et al., "Transient Functional Expression of Alphavbeta 3 on Vascular Cells During Wound Repair," *Am J Pathol,* 148(5):1407-21 (1996), the contents of which are incorporated herein by reference in their entirety.

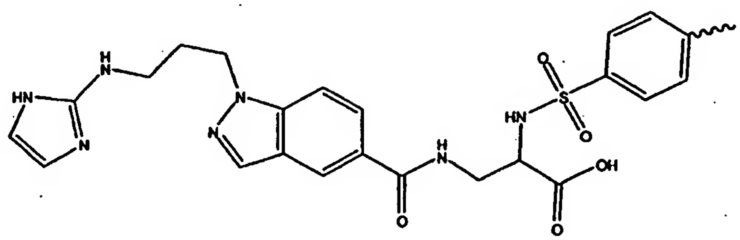
A large number of compounds are known that bind with high affinity and selectivity to $\alpha_v\beta_3$. The following references relate to this subject matter: Keenan

R.M., et al., "Benzimidazole Derivatives as Arginine Mimetics in 1,4-Benzodiazepine Nonpeptide Vitronectin Receptor (Alpha V Beta 3) Antagonists," *Bioorg Med Chem Lett*, 8(22):3165-70 (1998); Hart S.L., et al., "Cell Binding and Internalization by Filamentous Phage Displaying a Cyclic Arg-Gly-Asp-Containing Peptide," *J Biol Chem*, 269(17):12468-74 (1994); Keenan R.M., et al., "Conformational Preferences in a Benzodiazepine Series of Potent Nonpeptide Fibrinogen Receptor Antagonists," *J Med Chem*, 42(4):545-59 (1999); Nicolaou K.C., et al., "Design, Synthesis and Biological Evaluation of Nonpeptide Integrin Antagonists," *Bioorg Med Chem*, 6(8):1185-208 (1998); Keenan R.M., et al., "Discovery of Potent Nonpeptide Vitronectin Receptor (Alpha V Beta 3) Antagonists," *J Med Chem*, 40(15):2289-92 (1997); Bitan G., et al., " Design and Evaluation of Benzophenone-Containing Conformationally Constrained Ligands as Tools for Photoaffinity Scanning of the Integrin Alphavbeta3-Ligand

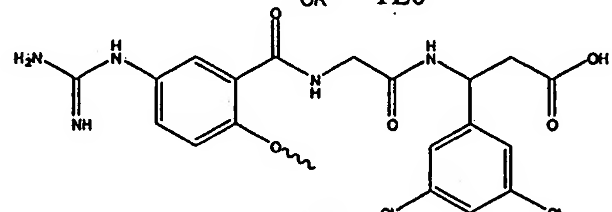
Bimolecular Interaction," *J Pept Res*, 55(3):181-94 (2000); Rockwell A.L., et al., "Rapid Synthesis of RGD Mimetics with Isoxazoline Scaffolds on Solid Phase: Identification of Alphavbeta3 Antagonists Lead Compounds," *Bioorg Med Chem Lett*, 9(7):937-42 (1999); Keenan R.M., et al., "Orally Bioavailable Nonpeptide Vitronectin Receptor Antagonists Containing 2-Aminopyridine Arginine Mimetics," *Bioorg Med Chem Lett*, 9(13):1801-6 (1999); Burgess K., et al., "Synthesis and Solution Conformation of Cyclo[RGDRGD]: a Cyclic Peptide with Selectivity for the Alpha V Beta 3 Receptor," *J Med Chem*, 39(22):4520-6 (1996); Yamada T., et al., "Tailoring Echistatin to Possess Higher Affinity for Integrin alpha(IIb)beta(3)," *FEBS Lett*, 387(1):11-15 (1996); WO 96-US13500, 1997, Ruminski P.G., et al., "Preparation of Meta-Guanidine, Urea, Thiourea or

Azacyclic Amino Benzoic Acid Derivatives as Integrin Antagonists"; Carron C.P., et al., "A Peptidomimetic Antagonist of the Integrin $\alpha v\beta 3$ Inhibits Leydig Cell Tumor Growth and the Development of Hypercalcemia of Malignancy," *Cancer Res,* 58(9):1930-1935 (1998), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to $\alpha_v\beta_3$. In preferred embodiments, (embodiments TL6, TL7, and TL8) the targeting ligand is comprised of one of the following structures:

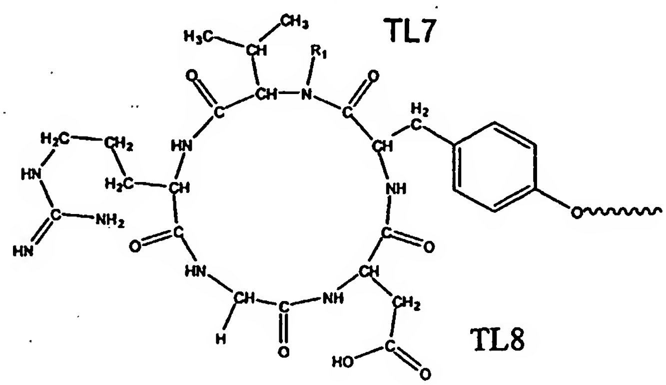


OR TL6



OR

TL7



TL8

wherein the wavy line is the site of linker attachment to the remainder of the drug complex and R_1 is H, or methyl, and amino acids in the cyclopeptide are the L-configuration except for the tyrosine which is the D-configuration.

In preferred embodiments of the invention the targeting ligand for $\alpha_V\beta_3$ is used in conjunction with targeting ligands that bind to other target receptors which are over-expressed on tumor neovasculature such as urokinase, plasmin, MMP-1-, MMP-3. MMP-9, membrane type –1 matrix metalloproteinase, or prostate specific membrane antigen.

Matrix Metalloproteinases as Targets

Matrix metalloproteases (MMP) are enzymes, which degrade connective tissue and which are over-expressed by a large number of tumors and stroma of tumors. There have been an enormous number of inhibitors to matrix metalloproteases developed as potential anti-cancer drugs. However, inhibition of MMP activity does not typically produce cytotoxicity and several clinical trials to date have failed to show efficacy of MMP inhibitors as antimetastatic drugs. At the present time, there are no known methods to convert the over-expression of MMPs into selective tumor toxicity. The following references relate to this subject matter: Nelson A.R., et al., "Matrix Metalloproteinases: Biologic Activity and Clinical Implications," *J Clin Oncol,* 18(5):1135 (2000); Whittaker M., et al., "Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors," *Chem Rev,* 99:2735-2776 (1999); Curran S.; Murray G.I., "Matrix Metalloproteinases in Tumour Invasion and Metastasis," *J Pathol,* 189(3):300-

308 (1999), the contents of which are incorporated herein by reference in their entirety.

Membrane type metalloproteinases are associated with the cell surface by a hydrophobic transmembrane domains or glycosylphosphatidylinositol anchors. Other MMP's become associated with the surface of tumor cells by a variety of mechanisms which include binding to:

1.) MT-1-MMP and TIMP2 (tissue inhibitor of metalloproteinase);
2.) Heparin sulfate proteoglycans;
3.) Hyaluronan receptor CD44;
4.) Integrin alpha V beta 3; and
5.) Extracellular matrix metalloproteinase inducer (EMMPRIN) specific receptors.

Accordingly, ligands, which bind to MMP's, can be employed in targeting tumors. The following references relate to this subject matter: Sato H., et al., "Cell Surface Binding and Activation of Gelatinase a Induced by Expression of Membrane-Type-1-Matrix Metalloproteinase (MT1-MMP)," *FEBS Lett,* 385(3):238-40 (1996); Monsky W.L., et al., "Binding and Localization of M(r) 72,000 Matrix Metalloproteinase at Cell Surface Invadopodia," *Cancer Res,* 53(13):3159-64 (1993); Yu Q; Stamenkovic I., "Cell Surface-Localized Matrix Metalloproteinase-9 Proteolytically Activates TGF-Beta and Promotes Tumor Invasion and Angiogenesis," *Genes Dev,* 14(2):163-76 (2000); Menashi S., et al., "Density-dependent Regulation of Cell-Surface Association of Matrix Metalloproteinase-2 (MMP-2) in Breast-Carcinoma Cells," *Int J Cancer,*

75(2):259-65 (1998); Deryugina E.I., et al., "Functional Activation of Integrin Alpha V Beta 3 in Tumor Cells Expressing Membrane-Type 1 Matrix Metalloproteinase," *Int J Cancer,* 86(1):15-23 (2000); Guo H., et al., "EMMPRIN (CD147), an Inducer of Matrix Metalloproteinase Synthesis, also Binds Interstitial Collagenase to the Tumor Cell Surface," *Cancer Res,* 60(4):888-91 (2000); Sawicki G., et al., "Expression of the Active Form of MMP-2 on the Surface of Leukemic Cells Accounts for their in Vitro Invasion," *J Cancer Res Clin Oncol,* 124(5):245-52 (1998); Yu W.H.; Woessner J.F. Jr., "Heparan Sulfate Proteoglycans as Extracellular Docking Molecules for Matrilysin (Matrix Metalloproteinase 7)," *J Biol Chem,* 275(6):4183-91 (2000); Chen W.T.; Wang J.Y., "Specialized Surface Protrusions of Invasive Cells, Invadopodia and Lamellipodia, have Differential MT1-MMP, MMP-2, and TIMP-2 Localization," *Ann NY Acad Sci,* 878:361-71 (1999); Brooks P.S., et al., "Localization of Matrix Metalloproteinase MMP-2 to the Surface of Invasive Cells by Interaction with Integrin Alpha V Beta 3," *Cell,* 85(5):683-93 (1996); Yu Q; Stamenkovic I., "Localization of Matrix Metalloproteinase 9 to the Cell Surface Provides a Mechanism for CD44-Mediated Tumor Invasion," *Genes Dev,* 13(1):35-48 (1999); Bourguignon L.Y., et al., "CD44v(3,8-10) is Involved in Cytoskeleton-Mediated Tumor Cell Migration and Matrix Metalloproteinase (MMP-9) Association in Metastatic Breast Cancer Cells," *J Cell Physiol,* 176(1):206-15 (1998); Corcoran M.L., et al., "TIMP-2 Mediates Cell Surface Binding of MMP-2," *Adv Exp Med Biol,"* 389:295-304 (1996); Emonard H.P., et al., "Tumor Cell Surface-Associated Binding Site for the M(R) 72,000 Type IV Collagenase," *Cancer Res,* 52(20):5845-8 (1992); Barmina O.Y., et al., "Collagenase-3 Binds to a Specific Receptor and Requires the Low Density Lipoprotein Receptor-

Related Protein for Internalization," *J Biol Chem,* 274(42):30087-93 (1999), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to a matrix metalloproteinase.

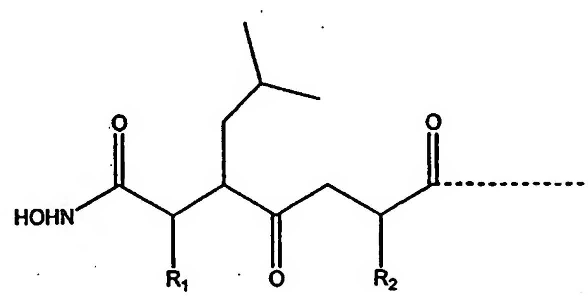
Matrix Metalloproteinase 7 Selective Ligands:

Matrix Metalloproteinase 7 (MMP-7 or Matrilysin) is a protease, which is constitutively produced by exocrine epithelial cells. MMP-7 is over-expressed by tumor cells in wide range of malignancies including: ovarian, gastric, prostate, colorectal, endometrial, gliomas, and breast cancer. MMP-7 contrasts with many other metalloproteases, which are over-expressed by tumor stromal elements rather than the tumor cells. At the present time there are no known methods to convert the over-expression of MMP-7 into selective tumor toxicity. The following references relate to this subject matter: Yamamoto H., et al., "Association of Matrilysin Expression with Recurrence and Poor Prognosis in Human Esophageal Squamous Cell Carcinoma," *Cancer Res,* 59(14):3313-6 (1999); Adachi Y., et al., "Contribution of Matrilysin (MMP-7) to the Metastatic Pathway of Human Colorectal Cancers," *Gut,* 45(2):252-8 (1999); Yamashita K, et al., "Expression and Tissue Localization of Matrix Metalloproteinase 7 (Matrilysin) in Human Gastric Carcinomas. Implications for Vessel Invasion and Metastasis," *Int J Cancer,* 79(2):187-94 (1998); Pacheco M.M., et al., "Expression of Gelatinases A and B, Stromelysin-3 and Matrilysin Genes in Breast Carcinomas:

Clinico-Pathological Correlations," *Clin Exp Metastasis,* 16(7):577-85 (1998); Hashimoto K., et al., "Expression of Matrix Metalloproteinase-7 and Tissue Inhibitor of Metalloproteinase-1 in Human Prostate," *J Urol,* 160(5):1872-6 (1998); Mori M., et al., "Over-expression of Matrix Metalloproteinase-7 mRNA in Human Colon Carcinomas," *Cancer,* 75(6 Suppl):1516-9 (1995); Honda M., et al., "Matrix Metalloproteinase-7 Expression in Gastric Carcinoma," *Gut,* 39(3):444-8 (1996); Nakano A., et al., "[Increased Expression of Gelatinases A and B, Matrilysin and TIMP-1 Genes in Human Malignant Gliomas]," *Nippon Rinsho,* 53(7):1816-21 (1995); Knox J.D., et al., "Matrilysin Expression in Human Prostate Carcinoma," *Mol Carcinog,* 15(1):57-63 (1996); Adachi Y., et al., "Matrix Metalloproteinase Matrilysin (MMP-7) Participates in the Progression of Human Gastric and Esophageal Cancers," *Int J Oncol,* 13(5):1031-5 (1998); Ueno H., et al., "Enhanced Production and Activation of Matrix Metalloproteinase-7 (Matrilysin) in Human Endometrial Carcinomas," *Int J Cancer,* 84(5):470-7 (1999); Barille S., et al., "Production of Metalloproteinase-7 (Matrilysin) by Human Myeloma Cells and its Potential Involvement in Metalloproteinase-2 Activation," *J Immunol,* 163(10):5723-8 (1999); Senota A., et al.," Relation of Matrilysin Messenger RNA Expression with Invasive Activity in Human Gastric Cancer," *Clin Exp Metastasis,* 16(4):313-21 (1998); Saarialho-Kere U.K., et al., "Matrix Metalloproteinase Matrilysin is Constitutively Expressed in Adult Human Exocrine Epithelium," *J Invest Dermatol,* 105(2):190-6 (1995); Tanimoto H., et al., "The Matrix Metalloprotease Pump-1 (MMP-7, Matrilysin): A Candidate Marker/Target for Ovarian Cancer Detection and Treatment," *Tumour Biol,* 20(2):88-98 (1999), the contents of which are incorporated herein by reference in their entirety.

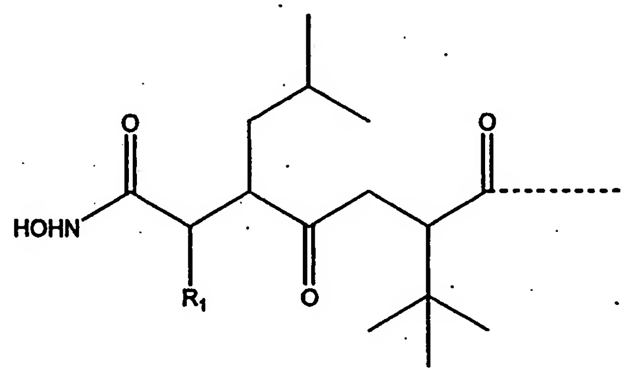
In a preferred embodiment, An is a ligand for MMP-7. A large number of potent reversible ligands are known that reversibly inhibit MMP-7. The following references relate to this subject matter: Whittaker M., et al., "Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors," *Chem Rev,* 99:2735-2776 (1999); Pratt L.M., et al., "The Synthesis of Novel Matrix Metalloproteinase Inhibitors Employing the Ireland-Claisen Rearrangement," *Bioorg Med Chem Lett,* 8:1359-1364 (1998); Abramson S.R., et al., "Characterization of Rat Uterine Matrilysin and Its cDNA," *J Biological Chem,* 270(27):16016-16022 (1995); Nelson A.R., et al., "Matrix Metalloproteinases: Biologic Activity and Clinical Implications," *J Clin Oncology,* 18(5):1135-1149 (2000), the contents of which are incorporated herein by reference in their entirety.

Preferred embodiment (embodiment TL9 and TL10) of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to MMP-7 comprised of the following structure:



TL9

wherein the dotted line is the site of attachment or linker attachment to the remainder of the drug complex and wherein R1 is hydroxy, methyl, ethyl, isopropyl, cyclopentyl, 3-(tetrahydrothiophenyl), or thiopen-2-ylthiomethyl-, and wherein R2 is benzyl, t-butyl, or isopropyl. These ligands can also bind to a number of other MMP's that are enriched in tumors.



TL10

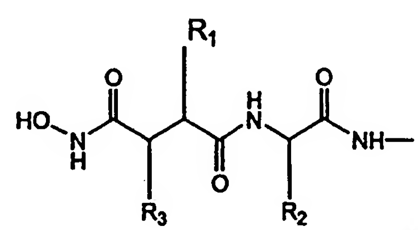
MMP1, 2, 3, 9 and Membrane Type 1 MMP. Targeting Ligands:

MMP 1, 2, 3, 9 and membrane type MMP 1(MT-MMP-1) are all over-expressed in a wide variety of malignancies. The following references relate to this subject matter: Stearns M.; Stearns M.E., "Evidence for Increased Activated Metalloproteinase 2 (MMP-2a) Expression Associated with Human Prostate Cancer Progression," *Oncol Res,* 8(2):69-75 (1996); Moll U.M., et al., "Localization of Collagenase at the Basal Plasma Membrane of a Human Pancreatic Carcinoma Cell Line," *Cancer Res,* 50(21):6995-70 (1990); Poulsom R., et al., "Expression of Gelatinase A and TIMP-2 mRNAs in Desmoplastic Fibroblasts in Both Mammary Carcinomas and Basal Cell Carcinomas of the Skin," *J Clin Pathol,* 46(5):429-36 (1993); Jones, J.L., et al., "Expression of MMP-2 and MMP-9, Their Inhibitors, and the Activator MT1-MMP in Primary

Breast Carcinomas," *J Pathol,* 189(2):161-168 (1999); Polette M., et al., "Gelatinase A Expression and Localization in Human Breast Cancers. An in Situ Hybridization Study and Immunohistochemical Detection using Confocal Microscopy," *Virchows Arch,* 424(6):641-5 (1994); Ohtani H., et al., "Immunoelectron Microscopic Localization of Gelatinase A in Human Gastrointestinal and Skin Carcinomas: Difference Between Cancer Cells and Fibroblasts," *Jpn J Cancer Res,* 86(3):304-9 (1995); Montironi R., et al., "Immunohistochemical Evaluation of Type IV Collagenase (72-Kd Metalloproteinase) in Prostatic Intraepithelial Neoplasia," *Anti-cancer Res,* 16(4A):2057-62 (1996); Stearns M.E.; Stearns M., "Immunohistochemical Studies of Activated Matrix Metalloproteinase-2 (MMP-2a)Expression in Human Prostate Cancer," *Oncol Res,* 8(2):63-7 (1996); Caudroy S., et al., "Expression of the Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) and the Matrix Metalloproteinase-2 in Bronchopulmonary and Breast Lesions," *J Histochem Cytochem,* 47:1575-1580 (1999); Montironi R., et al., "Location of 72-kd Metalloproteinase (Type IV Collagenase) in Untreated Prostatic Adenocarcinoma," *Pathol Res Pract,* 191(11):1140-6 (1995); Hamdy F.C., et al., "Matrix Metalloproteinase 9 Expression in Primary Human Prostatic Adenocarcinoma and Benign Prostatic Hyperplasia," *Br J Cancer,* 69(1):177-82 (1994); Nelson A.R., et al., "Matrix Metalloproteinases: Biologic Activity and Clinical Implications," *J Clin Oncol,* 18(5):1135 (2000); Emonard H.P. et al., "Tumor Cell Surface-Associated Binding Site for the M(R) 72,000 Type IV Collagenase," *Cancer Res,* 52(20):5845-8 (1992); Bramhall S.R., et al., "Imbalance of Expression of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of the Matrix Metalloproteinases (TIMPs) in Human Pancreatic

Carcinoma," *J Pathol,* 182(347-355 (1997); Väänen A., et al., "Prognostic Value of MMP-2 Immunoreactive Protein (72kD Type IV Collagenase) in Primary Skin Melanoma," *J Pathol,* 186:51-58 (1998); Murray G.I., et al., "Matrix Metalloproteinases and their Inhibitors in Gastric Cancer," *Gut,* 43(6):791-7 (1998); Lebeau A., et al., "Tissue Distribution of Major Matrix Metalloproteinases and their Transcripts in Human Breast Carcinomas," *Anti-cancer Res,* 19(5B):4257-64 (1999); Murray G.I., et al., "Matrix Metalloproteinase-1 is Associated with Poor Prognosis in Oesophageal Cancer," *J Pathol,* 185:256-261 (1998); Guo H., et al., "Emmprin (CD147), an Inducer of Matrix Metalloproteinase Synthesis, also Binds Interstitial Collagenase to the Tumor Cell Surface," *Cancer Res,* 60(4):888-91 (2000), the contents of which are incorporated herein by reference in their entirety.

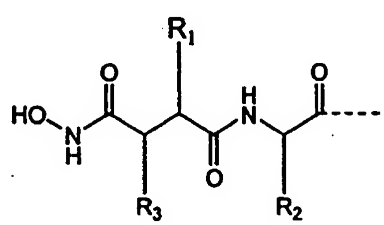
Similarities in the active site of these enzymes allow for targeting with a common family of ligands. Compounds of the following structure bind reversibly to MMP 1, 2, 3, 9 and membrane type MMP 1 with IC_{50} in the nanomolar to subnanomolar range.



wherein R_1 is $-CH_2CH(CH_3)_2$, $-(CH_2)_2C_6H_5$, $-(CH_2)_3C_6H_5$, n-butyl, n-hexyl, n-octyl, R_2 is C_6H_5, — C_6H_{11}, - $C(CH_3)_3$, (indol-3-yl)methyl, $-CH_2C_6H_5$, (5, 6, 7, 8 -terahydro-1-napthyl)methyl, $-CH(CH_3)_2$, 1-(napthyl)methyl, 3-(napthyl)methyl,

1-(quinolyl)methyl, 3-(quinolyl)methyl, 3-pyridylmethyl, 4-pyridylmethyl, t-butyl, and R_3 is H, OH, methyl, 2-thienylthiomethyl, or allyl. The following references relate to this subject matter: Yamamoto M., et al., "Inhibition of Membrane-Type 1 Matrix Metalloproteinase by Hydroxamate Inhibitors: An Examination of the Subsite Pocket," *J Med Chem*, 41:1209-1217 (1998); Curtin M.L., et al., "Broad Spectrum Matrix Metalloproteinase Inhibitors: An Examination of Succinamide Hydroxamate Inhibitors with P_1C_α Gem-Disubstitution," *Biorg Med Chem Lett*, 8:1443-1448 (1998); Levy D.E., et al., "Matrix Metalloproteinase Inhibitors: A Structure-Activity Study," *J Med Chem*, 41:199-223 (1998), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to MMP1, 2, 3, 9 or MT-MMP-1. In preferred embodiments, the targeting ligand comprises the following structure:

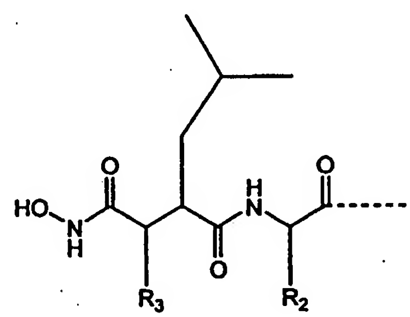


TL11

wherein the dotted line is the site of linker attachment to the remainder of the drug complex wherein R_1 is $-CH_2CH(CH_3)_2$, $-(CH_2)_2C_6H_5$, $-(CH_2)_3C_6H_5$, n-

butyl, n-hexyl, n-octyl, R_2 is C_6H_5, — C_6H_{11}, - $C(CH_3)_3$, (indol-3-yl)methyl, -$CH_2C_6H_5$, (5, 6, 7, 8 -terahydro-1-napthyl)methyl, –$CH(CH_3)_2$, 1-(napthyl)methyl, 3-(napthyl)methyl, 1-(quinolyl)methyl, 3-(quinolyl)methyl, 3-pyridylmethyl, 4-pyridylmethyl, t-butyl, and R_3 is H, OH, methyl, 2-thienylthiomethyl, or allyl.

In preferred embodiments (embodiment TL12), the targeting ligand comprises the following structures:



TL12

wherein R_2 is benzyl and R_3 is 2-thienylthiomethyl; or wherein R_2 is 5, 6, 7, 8,-terahydro-1-napthyl)methyl and R_3 is methyl; or wherein R_2 is t-butyl and R_3 is OH; or wherein R_2 is H and R_3 is (indol-3-yl)methyl; and wherein the dotted line is the site of linker attachment to the remainder of the drug complex.

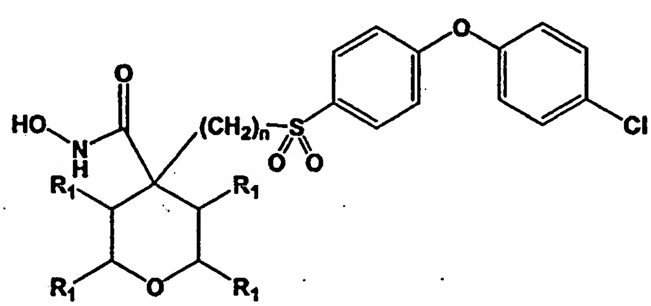
Another preferred embodiment is based on diphenlyether sulfone inhibitors of MMP's, which are highly active against MMP2, 3, 9, 12, and 13 MMP. The following references relate to this subject matter: 5,932,595, 8/03/99, Bender et al., "Matrix Metalloprotease Inhibitors"; Lovejoy B., et al., "Crystal Structures of MMP-1 and -13 Reveal the Structural Basis for Selectivity of Collagenase

Inhibitors," *Nat Struct Biol,* 6(3):217-21 (1999); Botos I., et al., "Structure of Recombinant Mouse Collagenase-3 (MMP-13)," *J Mol Biol,* 292:837-844 (1999), the contents of which are incorporated herein by reference in their entirety.

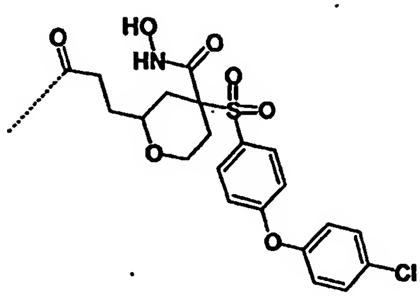
MMP 13 is an attractive target as it is over-expressed in a wide range of malignancies. The following references relate to this subject matter: Pendas A.M., et al., "An Overview of Collagenase-3 Expression in Malignant Tumors and Analysis of its Potential Value as a Target in Antitumor Therapies," *Clin Chim Acta,* 291(2):137-55 (2000); Shalinsky D.R., et al., "Broad Antitumor and Antiangiogenic Activities of AG3340, a Potent and Selective MMP Inhibitor Undergoing Advanced Oncology Clinical Trials," *Ann NY Acad Sci,* 878:236-70 (1999); Johansson N., et al., "Collagenase-3 (MMP-13) is Expressed by Tumor Cells in Invasive Vulvar Squamous Cell Carcinomas," *Am J Pathol,* 154(2):469-80 (1999); Barmina O.Y., et al., "Collagenase-3 Binds to a Specific Receptor and Requires the Low Density Lipoprotein Receptor-Related Protein for Internalization," *J Biol Chem,* 274(42):30087-93 (1999); Cazorla M., et al., "Collagenase-3 Expression is Associated with Advanced Local Invasion in Human Squamous Cell Carcinomas of the Larynx," *J Pathol,* 186(2):144-150 (1998); Balbin M., et al., "Expression and Regulation of Collagenase-3 (MMP-13) in Human Malignant Tumors," *APMIS,* 107(1):45-53 (1999); Johansson N., et al., "Expression of Collagenase-3 (Matrix Metalloproteinase-13) in Squamous Cell Carcinomas of the Head and Neck," *Am J Pathol,* 151(2):499-508 (1997); Uria J.A., et al., "Regulation of Collagenase-3 Expression in Human Breast Carcinomas is Mediated by Stromal-Epithelial Cell Interactions," *Cancer Res,* 57(21):4882-8 (1997); Airola K., et al., "Human Collagenase-3 is Expressed in

Malignant Squamous Epithelium of the Skin," *J Invest Dermatol,* 109:225-231 (1997); Freije J.M., et al., "Molecular Cloning and Expression of Collagenase-3, A Novel Human Matrix Metalloproteinase Produced by Breast Carcinomas," *J Biol Chem,* 269:24):16766-73 (1994); Uria J.A., et al., "Regulation of Collagenase-3 Expression in Human Breast Carcinomas is Mediated by Stromal-Epithelial Cell Interactions," *Cancer Res,* 57(2):4882-8 (1997), the contents of which are incorporated herein by reference in their entirety.

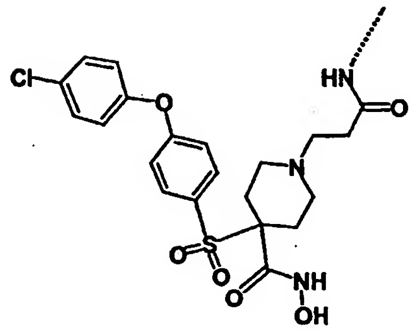
A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to MMP13. In preferred embodiments (TL13, TL14, and TL15), the targeting ligand comprises the following structure:



TL13



TL14



TL15

wherein n= 0 or 1 and wherein R_1 is H, or the site of linker attachment to the remainder of the drug complex, and the dotted line is the site of linker attachment to the remainder of ET.

Urokinase Selective Ligands:

Urokinase is a serine protease, which converts plasminogen into enzymatically active plasmin. The enzyme binds to specific cell surface receptors and is over-expressed in most major types of cancers. Hepatocyte growth factor/ Scatter Factor activation of the c-Met receptor, which is a characteristic of most malignancies, stimulates urokinase production. The overexpressioin of urokinase is a major adverse prognostic factor in multiple types of cancer including: breast, ovarian, prostate, colorectal, pancreatic, esophageal, gastric, renal, endometrial, and lung cancer. The expression of urokinase facilitates tissue invasion and metastasis. Depending upon the tumor type, urokinase can be located on tumor cells, stromal cells in the tumor, and on tumor-associated neovasculature. Urokinase, although an excellent marker of the malignant

phenotype, is not unique to malignancy. Urokinase is constitutively expressed in the eye, kidney, testes, and in atherosclerotic vessels. The following references relate to this subject matter: Duffy M.J., et al., "Urokinase Plasminogen Activator: A Prognostic Marker in Multiple Types of Cancer," *J Surg Oncol,* 71(2):130-5 (1999); Ploug M., et al., "Ligand Interaction between Urokinase-Type Plasminogen Activator and its Receptor Probed with 8-Anilino-1-Naphthalenesulfonate. Evidence for a Hydrophobic Binding Site Exposed only on the Intact Receptor," *Biochemistry,* 33(30):8991-7 (1994); Shiomi H., et al., "Cellular Distribution and Clinical Value of Urokinase-Type Plasminogen Activator, its Receptor, and Plasminogen Activator Inhibitor-2 in Esophageal Squamous Cell Carcinoma," *Am J Pathol,* 156(2):567-75 (2000); Harvey S.R., et al., "Demonstration of Urokinase Expression in Cancer Cells of Colon Adenocarcinomas by Immunohistochemistry and in Situ Hybridization," *Am J Pathol,* 155(4):1115-20 (1999); Bouchet C., et al., "Dissemination Risk Index Based on Plasminogen Activator System Components in Primary Breast Cancer," *J Clin Oncol,* 17(10):3048-57 (1999); Miyake H., et al., "Elevation of Urokinase-Type Plasminogen Activator and its Receptor Densities as New Predictors of Disease Progression and Prognosis in Men with Prostate Cancer," *Int J Oncol,* 14(3):535-41 (1999); Dubuisson L., et al., "Expression and Cellular Localization of the Urokinase-Type Plasminogen Activator and its Receptor in Human Hepatocellular Carcinoma," *J Pathol,* 190(2):190-5 (2000); Monvoisin A., et al., "Direct Evidence that Hepatocyte Growth Factor-Induced Invasion of Hepatocellular Carcinoma Cells is Mediated by Urokinase," *J Hepatol,* 30(3):511-8 (1999); Kobayashi H, et al., "Increased Cell-Surface Urokinase in Advanced Ovarian Cancer," *Jpn J Cancer Res,* 84(6):633-40 (1993); Yamamoto

M., et al., "Increased Expression of Low Density Lipoprotein Receptor-Related Protein/Alpha2-Macroglobulin Receptor in Human Malignant Astrocytomas," *Cancer Res,* 57(13):2799-805 (1997); Casslen B., et al., "Degradation of Urokinase Plasminogen Activator (UPA) in Endometrial Stromal Cells Requires both the UPA Receptor and the Low-Density Lipoprotein Receptor-Related Protein/Alpha2-Macroglobulin Receptor," *Mol Hum Reprod,* 4(6):585-93 (1998); Nykjaer A., et al., "Mannose 6-phosphate/insulin-like Growth Factor-II Receptor Targets the Urokinase Receptor to Lysosomes via a Novel Binding Interaction," *J Cell Biol,* 141(3):815-28 (1998); Noorman F., et al., "Degradation of Tissue-Type Plasminogen Activator by Human Monocyte- Derived Macrophages is Mediated by the Mannose Receptor and by the Low- Density Lipoprotein Receptor-Related Protein," *Blood,* 86(9):3421-7 (1995); Wohn K.D., et al., "The Urokinase-Receptor (CD87) is Expressed in Cells of the Megakaryoblastic Lineage," *Thromb Haemost,* 77(3):540-7 (1997); Volm M., et al., "Relationship of Urokinase and Urokinase Receptor in Non-Small Cell Lung Cancer to Proliferation, Angiogenesis, Metastasis and Patient Survival," *Oncol Rep,* 6(3):611-5 (1999); Foekens J.A., et al., "The Urokinase System of Plasminogen Activation and Prognosis in 2780 Breast Cancer Patients," *Cancer Res,* 60(3):636-43 (2000); Eatock, M.M., et al., "Tumour Vasculature as a Target for Anti-cancer Therapy," *Cancer Treat Rev,* 26(3):191-204 (2000); Conese M., et al., "alpha-2 Macroglobulin Receptor/Ldl Receptor-Related Protein(Lrp)-Dependent Internalization of the Urokinase Receptor," *J Cell Biol,* 131(6 Pt 1):1609-22 (1995); Duffy MJ, et al. "Urokinase Plasminogen Activator as a Predictor of Aggressive Disease in Breast Cancer," *Enzyme Protein,* 49(1-3):85-93 (1996); Fujii T., et al., "Urokinase-type Plasminogen Activator and

Plasminogen Activator Inhibitor-1 as a Prognostic Factor in Human Colorectal Carcinomas," *Hepatogastroenterology,* 46(28):2299-308 (1999); Brown P.A., et al., "Urokinase-Plasminogen Activator is Synthesized in Vitro by Human Glomerular Epithelial Cells but not by Mesangial Cells," *Kidney Int,* 45(1):43-7 (1994); Gunnarsson M., et al., "Factors of the Plasminogen Activator System in Human Testis, as Demonstrated by In-Situ Hybridization and Immunohistochemistry," *Mol Hum Reprod,* 5(10):934-40 (1999); Falkenberg M., et al., "Localization of Fibrinolytic Activators and Inhibitors in Normal and Atherosclerotic Vessels," *Thromb Haemost,* 75(6):933-8 (1996); Tripathi R.C., et al., "Localization of Urokinase-Type Plasminogen Activator in Human Eyes: An Immunocytochemical Study," *Exp Eye Res,* 51(5):545-52 (1990); Wagner S.N., et al., "Sites of Urokinase-Type Plasminogen Activator Expression and Distribution of its Receptor in the Normal Human Kidney," *Histochem Cell Biol,* 105(1):53-60 (1996), the contents of which are incorporated herein by reference in their entirety.

Since urokinase is such an important biochemical manifestation of the malignant phenotype, there have been extensive efforts to develop urokinase inhibitors and urokinase-targeted anti-cancer drugs. The following references relate to this subject matter: Jankun J., "Antitumor Activity of the Type 1 Plasminogen Activator Inhibitor and Cytotoxic Conjugate In Vitro," *Cancer Res,* 52(20):5829-32 (1992); Ke S.H., et al., "Optimal Subsite Occupancy and Design of a Selective Inhibitor of Urokinase," *J Biol Chem,* 272(33):20456-62 (1997); Ray P., et al., "Inhibitory Effect of Amiloride on the Urokinase Plasminogen Activators in Prostatic Cancer," *Tumour Biol,* 19(1):60-4 (1998); Yang S.Q., et al.,

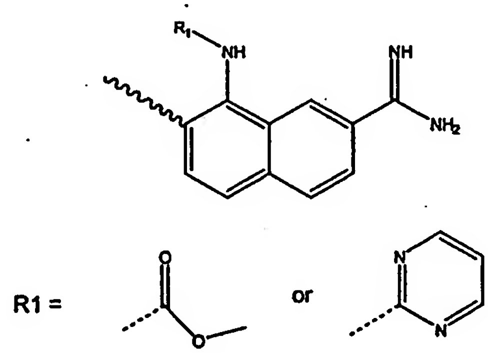
"Engineering Bidentate Macromolecular Inhibitors for Trypsin and Urokinase-Type Plasminogen Activator," *J Mol Biol,* 279(4):1001-11 (1998); Christova E., et al., "Hydrophobic Interactions in the Urokinase Active Centre. Inhibitory Action of Alkyl Ammonium and Amidinium Ions: Comparison with Trypsin," *Int J Pept Protein Res,* 15(5):459-63 (1980); Burgle M., et al., "Inhibition of the Interaction of Urokinase-Type Plasminogen Activator (uPA) with its Receptor (uPAR) by Synthetic Peptides," *Biol Chem,* 378(3-4):231-7 (1997); Towle M.J., et al., "Inhibition of Urokinase by 4-Substituted Benzo[B]Thiophene-2-Carboxamidines: An Important New Class of Selective Synthetic Urokinase Inhibitor," *Cancer Res,* 53(11):2553-9 (1993); Rabbani S.A., et al., "Prevention of Prostate-Cancer Metastasis In Vivo by a Novel Synthetic Inhibitor of Urokinase-Type Plasminogen Activator (uPA)," *Int J Cancer,* 63(6):840-5 (1995); Katz B.A., et al., "Structural Basis for Selectivity of a Small Molecule, S1-Binding, Submicromolar Inhibitor of Urokinase-Type Plasminogen Activator," *Chem Biol,* 7(4):299-312 (2000); Bridges A.J., et al., "The Synthesis of Three 4-Substituted BenzoThiophene-2-Carboxamidines as Potent and Selective Inhibitors of Urokinase," *Bioorg Med Chem,* 1(6):403-10 (1993); Billstrom A., et al., "The Urokinase Inhibitor P-Aminobenzamidine Inhibits Growth of a Human Prostate Tumor in SCID Mice," *Int J Cancer,* 61(4):542-7 (1995); Evans D.M., et al., "Time and Dose Dependency of the Suppression of Pulmonary Metastases of Rat Mammary Cancer by Amiloride," *Clin Exp Metastasis,* 16(4):353-7 (1998); Min H.Y., et al., "Urokinase Receptor Antagonists Inhibit Angiogenesis and Primary Tumor Growth in Syngeneic Mice," *Cancer Res,* 56(10):2428-33 (1996); Fibbi G., et al., "Urokinase-Dependent Angiogenesis In Vitro and Diacylglycerol Production are Blocked by Antisense Oligonucleotides against the Urokinase

Receptor," *Lab Invest,* 78(9):1109-19 (1998); Benzo[b]thiophene-2-carboxamidines: An Important New Class of Selective Synthetic Urokinase Inhibitor," *Cancer Res,* 53(11):2553-9 (1993); 5,656,726, 8/12/97, Rosenberg, et al., "Peptide Inhibitors of Urokinase Receptor Activity"; Rabbani SA, et al., "Prevention of Prostate-Cancer Metastasis In Vivo by a Novel Synthetic Inhibitor of Urokinase-Type Plasminogen Activator (uPA)," *Int J Cancer,* 63(6):840-5 (1995); Billstrom A., et al., "The Urokinase Inhibitor p-Aminobenzamidine Inhibits Growth of a Human Prostate Tumor in SCID Mice," *Int J Cancer,* 61(4):542-7 (1995); 5,747,458, 5/5/98, Rosenberg, et al., "Urokinase Receptor Ligands"; Schmitt M., "Urokinase-Type Plasminogen Activator (uPA) and its Receptor (CD87): A New Target in Tumor Invasion and Metastasis," *J Obstet Gynaecol,* 21(2):151-65 (1995); 5,679,350, 10/21/97, Jankun, et al., "Method of Delivery of a Medicament to a cancer Cell using a Pathway of Plasminogen Activator Material"; 5,552,390, 9/03/96, Scholar, et al., "Phosphorothioate Inhibitors of Metastatic Breast Cancer"; 5,519,120, 5/21/96, Dano, et al., "Urokinase-type Plasminogen Activator Receptor Antibodies"; 5,902,812, 5/11/99, Brocchini, et al., "Pharmaceutical Piperazine Compounds"; 5,891,877, 4/06/99, Brocchini, et al., "Pharmaceutical Compounds"; 5,750,530, 5/12/98, Bryans, et al., "Pharmaceutical Diketopiperazine Compounds"; 5,700,804, 12/23/97, Collins, et al., "Pharmaceutical Compounds"; 5,550,213, 8/27/96, Anderson, et al., "Inhibitors of Urokinase Plasminogen Activator"; 5,314,994, 5/24/94, Loskutoff, et al., "Inhibitor of Tissue-type and Urokinase-type Plasminogen Activators"; 5,340,833, 8/23/94, Bridges, et al., "Urokinase Inhibitors", the contents of which are incorporated herein by reference in their entirety.

An especially potent class of reversible urokinase inhibitors is naphthamidines, which are active inhibitors at nanomolar levels. The following references relate to this subject matter: Nienaber V.L., et al., "Structure-Directed Discovery of Potent Non-Peptidic Inhibitors of Human Urokinase that Access a Novel Binding Subsite," *Structure Fold Des,* 8(5):553-563 (2000), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to urokinase.

In preferred embodiments (TL16, TL17), the targeting ligand comprises the following structure:



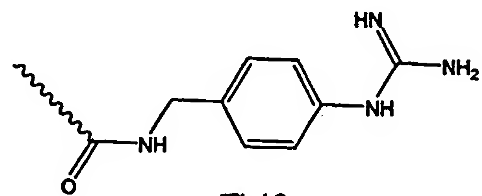
TL16 TL17

wherein the wavy line is the site of linker attachment to the remainder of the drug complex and the dotted line is the site of attachment of R_1.

Another preferred embodiment is based on the ability of phenylguanidines to inhibit urokinase. The following references relate to this subject matter: Sperl S., et al., "(4-Aminomethyl)Phenylguanidine Derivatives as Nonpeptidic Highly

Selective Inhibitors of Human Urokinase," *Proc Natl Acad Sci USA*, 97(10):5113-5118 (2000), the contents of which is incorporated herein by reference in its entirety.

In preferred embodiment (TL19), the targeting ligand comprises the following structure:

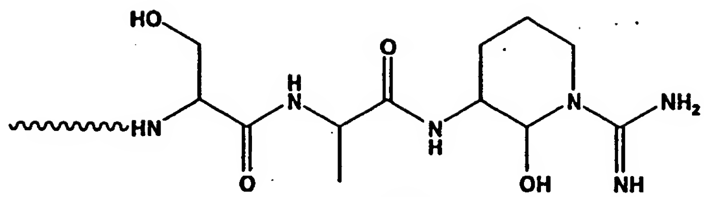


TL19

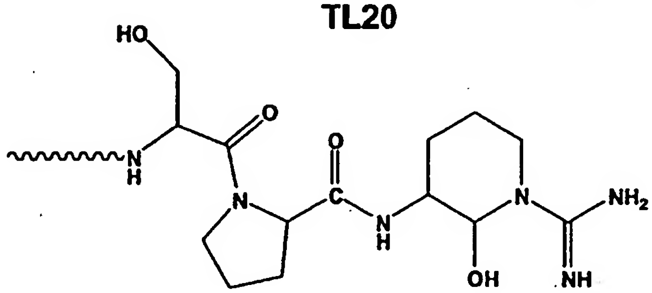
wherein the wavy line is the site of linker attachment to the remainder of the drug complex. In a preferred embodiment, ET has two groups of the structure shown above.

Another class of urokinase selective ligands is based on arginine aldehyde derivatives, which bind reversibly to urokinase with nanomolar affinity. The following references relate to this subject matter: Tamura S.Y., et al., "Synthesis and Biological Activity of Peptidyl Aldehyde Urokinase Inhibitors," *Bioorg Med Chem Lett*, 10:983-987 (2000), the contents of which is incorporated herein by reference in its entirety.

In preferred embodiment (TL20 and TL21), the targeting ligand comprises the following structure:



TL20



TL21

wherein the wavy line is the site of linker attachment to the remainder of the drug complex, and the serine residue has the D-configuration and the remainder of the amino acid residues has the L-configuration; or wherein the structures are L, D, or a racemic mixture.

Additional urokinase binding ligands are described in the neoantigen section that also comprise targeting ligands for urokinase.

Plasmin Selective Ligands:

As discussed above, many types of malignancies are characterized by high levels of urokinase and tissue plasminogen activator, which converts plasminogen into plasmin. Adenocarcinoma cells of the breast, colon and malignant osteoscarcoma bind large quantities of plasminogen and plasmin on the cell surface (10^5 to 5×10^7 molecules/cell). The membrane binding facilitates activation of the plasminogen into plasmin. Plasmin binds approximately 80

times tighter than plasminogen. In addition, membrane bound plasmin is resistant to inactivation by α_2 antiplasmin and α_2-macroglobulin. Cytokeratin 8 is the major plasminogen receptor on carcinoma cells. Plasminogen also binds to cell surface annexin II on tumors. Plasminogen is widely distributed throughout the body with plasma concentrations of approximately 1-2 micromolar. However, proteolytically active plasmin is tightly regulated and inhibited by a variety of naturally occurring protease inhibitors. Plasmin plays an important physiological role in fibrinolysis, wound healing, and ovulation. Congenital deficiency of plasminogen is characterized by the development of fibrinous conjunctivitis. The following references relate to this subject matter: Hembrough TA, et al., "A Cytokeratin 8-Like Protein with Plasminogen-Binding Activity is Present on the External Surfaces of Hepatocytes, HepG2 Cells and Breast Carcinoma Cell Lines," *J Cell Sci.*, 108 (Pt 3):1071-82 (1995); Campbell PG, et al., "Binding and Activation of Plasminogen on the Surface of Osteosarcoma Cells," *J Cell Physiol*, 159(1):1-10 (1994); Hembrough TA, et al., "Cell-Surface Cytokeratin 8 is the Major Plasminogen Receptor on Breast Cancer Cells and is Required for the Accelerated Activation of Cell-Associated Plasminogen by Tissue-Type Plasminogen Activator," *J Biol Chem*, 271 (41): 25684-91 (1996); Hembrough TA, et al., "Cytokeratin 8 Released by Breast Carcinoma Cells In Vitro Binds Plasminogen and Tissue-Type Plasminogen Activator and Promotes Plasminogen Activation," *Biochem J.*, 317(Pt 3): 763-9 (1996); Clavel C., et al., "Detection of The Plasmin System in Human Mammary Pathology Using Immunofluorescence," *Cancer Res.*, 46(11):5743-7 (1986); Ranson M., et al., "Increased Plasminogen Binding is Associated with Metastatic Breast Cancer Cells: Differential Expression of Plasminogen Binding Proteins," *Br J Cancer*,

77(10):1586-97 (1998); Costantini V., et al., "Occurrence of Components of Fibrinolysis Pathways In Situ in Neoplastic and Nonneoplastic Human Breast Tissue," *Cancer Res*, 51(1):354-8 (1991); Gonzalez-Gronow M., et al., "Plasmin Binding to the Plasminogen Receptor Enhances Catalytic Efficiency and Activates the Receptor for Subsequent Ligand Binding," *Arch Biochem Biophys*, 286(2):625-8 (1991); Burtin P; Fondaneche MC., "Receptor for plasmin on human carcinoma cells," *J Natl Cancer Inst*, 80(10): 762-5 (1988); Miles LA, et al., "Role of Cell-Surface Lysines in Plasminogen Binding to Cells: Identification of Alpha-Enolase as a Candidate Plasminogen Receptor," *Biochemistry*, 30(6):1682-91 (1991); Burtin P., et al., "The Plasmin System in Human Adenocarcinomas and their Metastases. A Comparative Immunofluorescence Study," *Int J Cancer*, 39(2):170-8 (1987); Burtin P., et al., "The Plasmin System in Human Colonic Tumors: An Immunofluorescence Study," *Int J Cancer*, 35(3):307-14 (1985); Plow EF, et al., "The Plasminogen System and Cell Surfaces: Evidence for Plasminogen and Urokinase Receptors on the Same Cell Type," *J Cell Biol*, 103(6 Pt 1):2411-20 (1986); Kwaan HC, "The Plasminogen-Plasmin System in Malignancy," *Cancer Metastasis Rev*, 11(3-4):291-311 (1992); Correc P., et al., "The Presence of Plasmin Receptors on Three Mammary Carcinoma MCF-7 Sublines.," *Int J Cancer*, 46(4):745-50 (1990); Correc P., et al., "Visualization of the Plasmin Receptor on Carcinoma Cells," *Int J Cancer*, 50(5):767-71 (1992), the contents of which are incorporated herein by reference in their entirety.

There have been attempts to utilize the enhanced plasmin activity of tumor cells to activate plasmin selective cytotoxic prodrugs. Plasminogen activators have

been employed to target malignant cells by coupling cytotoxic agents to the protein plasminogen activator inhibitor types 1 and 2. However, tumor-associated plasmin has not been utilized as a target site for the selective delivery of anti-cancer drugs. The following references relate to this subject matter: Chakravarty PK, et al., "Plasmin-Activated Prodrugs for Cancer Chemotherapy. 1. Synthesis and Biological Activity of Peptidylacivicin and Peptidylphenylenediamine Mustard," *J Med Chem*, 26(5):633-8 (1983); Chakravarty PK, et al., "Plasmin-Activated Prodrugs for Cancer Chemotherapy. 2. Synthesis and Biological Activity of Peptidyl Derivatives of Doxorubicin," *J Med Chem*, 26(5):638-44 (1983); Abaza MS, et al., "Anti-Urokinase-Type Plasminogen Activator Monoclonal Antibodies Inhibit the Proliferation of Human Breast Cancer Cell Lines In Vitro," *Tumour Biol*, 19(4):229-37 (1998); Towle MJ, et al., "Inhibition of Urokinase by 4-Substituted."; 5,679,350, 10/21/97, Jankun, et al., "Method of Delivery of a Medicament to a Cancer Cell using a Pathway of Plasminogen Activator Material.", the contents of which are incorporated herein by reference in their entirety.

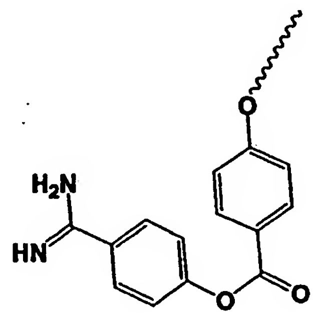
Plasmin is serine protease with broad substrate specificity for cleaving amide bond adjacent to lysine or arginine. P-amidinophenol esters are potent inhibitors of plasmin. These derivatives are inverse substrates and acylate a serine hydroxy group in the active site of the enzyme. If the acyl enzyme intermediate is sufficiently stable, irreversible inactivation of enzyme activity results. The p-amidinophenol ester of p-methoxybenzoic acid irreversibly inactivates plasmin. The following references relate to this subject matter: Nozawa M., et al., "Behavior of Trypsin and Related Enzymes Toward Amidinophenyl Esters," *J*

Pharmacobiodyn, 4(8):559-64 (1981); Nozawa M., et al., "Comparative Studies on the Structure of Active Sites. Behavior of "Inverse Substrates" Toward Trypsin and Related Enzymes," *J Biochem (Tokyo)*, 91(6):1837-43 (1982); Yamada H., et al., "Differentiation of Tryptic Enzymes Based on Enantiomeric Specificity at the Deacylation Step," *FEBS Lett*, 227(2):195-7 (1988); McLaren AB; Tanizawa K., "Deacylation Rate Constants of Acylated Human and Porcine Plasmins," *Aust. J. Biol. Sci.*, 37:205-10 (1984); Tanizawa K., et al., " "Inverse Substrates" for Trypsin. Efficient Enzymatic Hydrolysis of Certain Esters with a Cationic Center in the Leaving Group," *J Amer. Chem Soc.*, 99(13):4485-4488 (1977); Turner AD, et al., "*p*-Amidino Esters as Irreversible Inhibitors of Factors IXa and Xa and Thrombin," *Biochemistry*, 25:4929-4935 (1986); Fujioka T., et al., "Analysis of Latent Properties of Trypsin. Acyl Trypsins Derived from Enantiomeric Pairs of "Inverse Substrates"," *J Biochem.*, 89:637-643 (1981); Lynas J., et al., "Peptidyl Inverse Esters of p-Methoxybenzoic Acid: A Novel Class of Potent Inactivator of the Serine Proteases," *Biochem J.*, 325 (Pt 3):609-16 (1997); Fujioka T., et al., "Analysis of Latent Properties of Trypsin. Acyl Trypsins Derived from Enantiomeric Pairs of "Inverse Substrates"", *J Biochem (Tokyo)*, 89(2):637-43 (1981), the contents of which are incorporated herein by reference in their entirety.

By attaching a linker to this class of inhibitors, it is possible to chemically couple or target molecules to plasmin. This serves as the basis for being able to employ plasmin as a targeting entity.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to plasmin.

In preferred embodiment (TL22), the targeting ligand comprises the following structure:



TL22

wherein the wavy line is the site of the linker attachment to the remainder of the drug. Other preferred plasmin binding ligands are described in the neoantigen section that can be targeting ligands.

Cathepsin B Targeting Ligands

Cathepsin B is over-expressed and a major adverse prognostic factor in many human malignancies. Cathepsin B binds to annexin II on the surface of tumor cells. The following reference relate to this subject matter: Mai J., et al., "Cell Surface Complex of Cathepsin B/Annexin II Tetramer in Malignant Progression," *Biochimica Biophysica Acta (BBA)-Protein Molecular Enzymology,* 1477(1-2):215-230 (2000), the contents of which is incorporated herein by reference in its entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to Cathepsin B. This is discussed in more detail in the neoantigen section.

Matripase, Seprase, and Fibroblast Activation Protein Targeting Ligands

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to seprase, or fibroblast activation protein. This is discussed in more detail in the neoantigen section.

Prostate Specific Membrane Antigen Targeting Ligands:

Prostatic adenocarcinoma cells have high concentrations of the enzyme Glutamate Carboxypeptidase II or Prostatic Specific Membrane Antigen (PSMA) on the cell surface. In addition, the enzyme is present on the brush border of the kidneys, the luminal surface of parts of the proximal small intestine and in the brain. Radiolabelled monoclonal antibodies against PSMA (ProstaScint ™) are in clinical use to assess metastatic tumor spread. PSMA has also been detected on the surface of tumor neovasculature. Inhibitors of PSMA have been described as anti-cancer drugs. However, the activity of these compounds is weak and unlikely to be of clinical utility. PSMA positive human prostate tumor cells readily grow in vitro in the presence of high concentrations of 2-(phosphonomethyl)pentanedioic acid, a potent inhibitor of PSMA (A. Glazier, unpublished observations). Efforts are also underway to utilize PSMA related peptides as vaccines against prostate cancer. The following references relate to

this subject matter: US Patent 5,804,602, 9/8/98, Slusher, et al., "Methods of Cancer Treatment Using NAALADase Inhibitors"; US Patent 5,795,877, 8/18/98, Jackson, et al., "Inhibitors of NAALADase Enzyme Activity"; Murphy GP, et al., "Current Evaluation of the Tissue Localization and Diagnostic Utility of Prostate Specific Membrane Antigen," *Cancer*, 83(11):2259-69 (1998); Heston WD, "Characterization and Glutamyl Preferring Carboxypeptidase Function of Prostate Specific Membrane Antigen: A Novel Folate Hydrolase," *Urology*, 49(3A Suppl):104-12 (1997); Tiffany CW, et al., "Characterization of the Enzymatic of PSM: Comparison with Brain NAALADase [In Process Citation]," *Prostate*, 39(1):28-35 (1999); Murphy GP, et al., "Comparison of Serum PSMA, PSA Levels with Results of Cytogen-356 Prostascint Scanning in Prostatic Cancer Patients," *Prostate*, 33(4):281-5 (1997); Serval V. et al., "Competitive Inhibition of N-Acetylated-Alpha-Linked Acidic Dipeptidase Activity by N-Acetyl-L-Aspartyl-Beta-Linked L-Glutamate," *J Neurochem*, 55(1):39-46 (1990); Liu H. et al., "Constitutive and Antibody-Induced Internalization of Prostate-Specific Membrane Antigen," *Cancer Res*, 58(18):4055-60 (1998); Murphy GP, et al., "Current Evaluation of the Tissue Localization and Diagnostic Utility of Prostate Specific Membrane Antigen," *Cancer*, 83(11):2259-69 (1998); Jackson PF, et al., "Design, Synthesis, and Biological Activity of a Potent Inhibitor of the Neuropeptidase N-Acetylated Alpha-Linked Acidic Dipeptidase," *J Med Chem*, 39(2):619-22 (1996); Troyer JK; Beckett ML; Wright GL Jr., "Detection and Characterization of the Prostate-Specific Membrane Antigen (PSMA) in Tissue Extracts and Body Fluid," *Int J Cancer*, 62(5):552-8 (1995); Douglas TH, et al., "Effect of Exogenous Testosterone Replacement on Prostate-Specific Antigen and Prostate-Specific Membrane Antigen Levels in Hypogonadal Men," *J Surg*

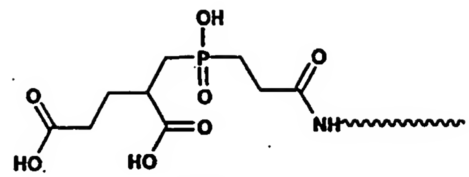
Oncol, 59(4):246-50 (1995); Kawakami M; Nakayama J., "Enhanced Expression of Prostate-Specific Membrane Antigen Gene in Prostate Cancer as Revealed by in Situ Hybridization," *Cancer Res,* 57(12):2321-4 (1997); Israeli RS, et al., "Expression of the Prostate-Specific Membrane Antigen," *Cancer Res,* 54(7):1807-11 (1994); Halsted CH, et al., "Folylpoly-Gamma-Glutamate Carboxypeptidase from Pig Jejunum. Molecular Characterization and Relation to Glutamate Carboxypeptidase II," *J Biol Chem,* 273(32):20417-24 (1998); Luthi-Carter R. et al., "Hydrolysis of The Neuropeptide N-Acetylaspartylglutamate (NAAG) by Cloned Human Glutamate Carboxypeptidase II," *Brain Res,* 795(1-2):341-8 (1998); Grauer LS, et al., "Identification, Purification, and Subcellular Localization of Prostate- Specific Membrane Antigen PSM' Protein in the LNCaP Prostatic Carcinoma Cell Line," *Cancer Res,* 58(21):4787-9 (1998); Slusher BS; Tsai G; Yoo G; Coyle JT, "Immunocytochemical Localization of the N-Acetyl-Aspartyl-Glutamate (NAAG) Hydrolyzing Enzyme N-Acetylated Alpha-Linked Acidic Dipeptidase (Naaladase)," *J Comp Neurol,* 315(2):217-29 (1992); Luthi-Carter R. et al., "Isolation and Expression of a Rat Brain cDNA Encoding Glutamate Carboxypeptidase II," *Proc Natl Acad Sci,* 95(6):3215-20 (1998); Troyer J.K., et al., "Location of Prostate-Specific Membrane Antigen in the LNCaP Prostate Carcinoma Cell Line," *Prostate,* 30(4):232-42 (1997); Sweat S.D, et al., "Prostate-Specific Membrane Antigen Expression is Greatest in Prostate Adenocarcinoma and Lymph Node Metastases," *Urology,* 52(4):637-40 (1998); Luthi-Carter R., et al., "Molecular Characterization of Human Brain N-Acetylated Alpha-Linked Acidic Dipeptidase (Naaladase)," *J Pharmacol Exp Ther,* 286(2):1020-5 (1998); Bzdega T. et al., "Molecular Cloning of a Peptidase Against N-Acetylaspartylglutamate from a Rat Hippocampal cDNA Library," *J*

Neurochem, 69(6):2270-7 (1997); Liu H. et al., "Monoclonal Antibodies to the Extracellular Domain of Prostate-Specific Membrane Antigen also React with Tumor Vascular Endothelium," *Cancer Res*, 57(17):3629-34 (1997); Barren RJ 3[rd]. et al., "Monoclonal Antibody 7E11.C5 Staining of Viable LNCaP Cells," *Prostate*, 30(1):65-8 (1997); Kawamata H. et al., "Active-MMP2 in Cancer Cell Nests of Oral Cancer Patients: Correlation with Lymph Node Metastasis," *Int J Oncol*, 13(4):699-704 (1998); Weissensteiner T., "Prostate Cancer Cells Show a Nearly 100-Fold Increase in the Expression of the Longer of Two Alternatively Spliced mRNAs of the Prostate- Specific Membrane Antigen (PSM) [letter]," *Nucleic Acids Res*, 26(2):687 (1998); Bostwick DG, et al., "Prostate Specific Membrane Antigen Expression in Prostatic Intraepithelial Neoplasia and Adenocarcinoma: A Study of 184 Cases," *Cancer*, 82(11):2256-61 (1998); Pinto JT, et al., "Prostate-Specific Membrane Antigen: A Novel Folate Hydrolase in Human Prostatic Carcinoma Cells," *Clin Cancer Res*, 2(9):1445-51 (1996); Silver DA, et al., "Prostate-Specific Membrane Antigen Expression in Normal and Malignant Human Tissues," *Clin Cancer Res*, 3(1):81-5 (1997); Sweat SD, et al., "Prostate-Specific Membrane Antigen Expression is Greatest in Prostate Adenocarcinoma and Lymph Node Metastases," *Urology*, 52(4):637-40 (1998); Carter R.E., et al., "Prostate-Specific Membrane Antigen is a Hydrolase with Substrate and Pharmacologic Characteristics of a Neuropeptidase," *Proc Natl Acad Sci*, 93(2):749-53 (1996); Fair W.R.; et al., "Prostate-Specific Membrane Antigen," *Prostate*, 32(2):140-8 (1997); Slusher BS, et al., "Rat Brain N-Acetylated Alpha-Linked Acidic Dipeptidase Activity. Purification and Immunologic Characterization," J *Biol Chem*, 265(34):21297-301 (1990); Subasinghe N. et al., "Synthesis Of Acyclic And Dehydroaspartic Acid Analogues

of Ac-Asp-Glu- OH and Their Inhibition of Rat Brain N-acetylated Alpha-linked Acidic Dipeptidase (NAALA Dipeptidase)," *J Med Chem*, 33(10):2734-44 (1990); Stauch BL, et al., "The Effects of N-acetylated Alpha-linked Acidic Dipeptidase (NAALADase) Inhibitors on [3H]NAAG Catabolism In Vivo," *Neurosci Lett*, 100(1-3):295-300 (1989); Berger U.V. et al., "The Immunocytochemical Localization of N-acetylaspartyl glutamate, its Hydrolysing Enzyme NAALADase, and the NMDAR-1 Receptor at a Vertebrate Neuromuscular Junction," *Neuroscience*, 64(4):847-50 (1995); Wright GL Jr. et al., "Upregulation of Prostate-Specific Membrane Antigen after Androgen- Deprivation Therapy," *Urology*, (2):326-34 (1996); Chang S.S., et al., "Prostate-specific Membrane Antigen is Produced in Tumor-associated Neovasculature," *Clin Cancer Res*, 5:2674-2681 (1999). Tjoa B.A., et al., "Follow-Up Evaluation of a Phase II Prostate Cancer Vaccine Trial," *Prostate*, 40(2):125-9 (1999), the contents of which are incorporated herein by reference in their entirety.

PSMA is a zinc carboxypeptidase, which catalyzes the hydrolysis of N-acetyl-aspartylglutamate and gamma glutamates. The enzyme is potently inhibited by phosphorous based transition state analogs. 2-(phosphonomethyl)-pentanedioic acid inhibits the enzyme with a Ki of 0.3 nanomolar. As described later in Example 1, it is possible to attach a linker to compounds of this class and retain inhibitory and enzyme binding capacity.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to PSMA. In a preferred embodment, the targeting ligand comprises the following structure:



TL23

wherein the wavy line is the site of linker attachment to the remainder of the drug complex.

The scope of the present invention also encompasses a ligand-linker complex that binds to PSMA and the use of this ligand-linker complex to target drugs to PSMA positive cells wherein the ligand is not a monoclonal antibody. The present invention also includes an anti-cancer drug comprised of the structure shown above covalently linked to a cytotoxic drug or cytotoxic agent.

Sigma Receptor Targeting Ligands

Sigma receptors are a class of membrane associated receptors, that are present in increased amounts on a variety of malignant tumors including: prostatic adenocarcinoma, neuroblastoma, melanoma, breast carcinoma, pheochromocytoma, renal carcinoma, colon carcinoma, and lung carcinoma. Prostatic adenocarcinoma cells have approximately 2 million receptors molecules/cells. Sigma receptors are also present on a variety of normal tissues including the liver, brain, kidney, and endocrine glands. Radiolabelled sigma receptor ligands concentrate in malignant tumors in vivo and have been described as tumor imaging agents. However, sigma receptors have not previously been exploited for targeting antineoplastic drugs. By themselves,

sigma receptors are unlikely to have sufficient tumor selectivity for tumor targeting purposes. However, sigma receptor targeting by a multifunctional delivery vehicle, which jointly targets other receptors enriched on tumor cells, can provide excellent tumor specificity. The following references relate to this subject matter: John CS, et al., "99mTc-labeled Sigma-Receptor-Binding Complex: Synthesis, Characterization,and Specific Binding to Human Ductal Breast Carcinoma (T47D) Cells," *Bioconjug Chem*, 8(3):304-9 (1997); John CS, et al., "A Malignant Melanoma Imaging Agent: Synthesis, Characterization, In Vitro Binding and Biodistribution of Iodine-125-(2-piperidinylaminoethyl)4-iodobenzamide," *J Nucl Med*, 34(12):2169-75 (1993); John CS, et al., "Synthesis, In Vitro Binding, and Tissue Distribution of Radioiodinated 2-[125I]N-(N-benzylpiperidin-4-yl)-2-iodo benzamide, 2-[125I]BP: A Potential Sigma Receptor Marker for Human Prostate Tumors," *Nucl Med Biol*, 25(3):189-94 (1998); John CS, et al., "An Improved Synthesis of [125I]N-(diethylaminoethyl)-4-iodobenzamide: A Potential Ligand for Imaging Malignant Melanoma," *Nucl Med Biol*, 20(1):75-9 (1993); Zhang Y. et al., "Characterization of Novel N,N'-disubstituted Piperazines as Sigma Receptor Ligands," *J Med Chem*, 41(25):4950-7 (1998); Vilner BJ, et al. "Cytotoxic Effects of Sigma Ligands: Sigma Receptor-Mediated Alterations in Cellular Morphology and Viability," *J Neurosci*, 15(1 Pt 1):117-34 (1995); Waterhouse RN, et al., "Examination of Four 123I-labeled Piperidine-Based Sigma Receptor Ligands as Potential Melanoma Imaging Agents: Initial Studies in Mouse Tumor Models," *Nucl Med Biol*, 24(6):587-93 (1997); John CS, et al., "Synthesis and Pharmacological Characterization of 4-[125I]-N-(N-benzylpiperidin-4-yl)-4-iodobenzamide: A High Affinity Sigma Receptor Ligand for Potential Imaging of Breast Cancer," *Cancer*

Res, 55(14):3022-7 (1995); Zamora PO; Moody TW; John CS, "Increased Binding to Sigma Sites of N-[1'(2-piperidinyl)ethyl)-4-[I- 125]-iodobenzamide (I-125-PAB) With Onset of Tumor Cell Proliferation," *Life Sci*, 63(18):1611-8 (1998); Bem WT, et al., "Over-expression of Sigma Receptors in Nonneural Human Tumors," *Cancer Res*, 51(24):6558-6 (1991); Mach RH, et al. "Sigma 2 Receptors as Potential Biomarkers of Proliferation in Breast Cancer," *Cancer Res*, 57(1):156-61 (1997); Brent PJ, et al. "Sigma Binding Site Ligands Inhibit Cell Proliferation in Mammary and Colon Carcinoma Cell Lines and Melanoma Cells in Culture," *Eur J Pharmacol*, 278(2):151-60 (1995); John CS, et al., "Sigma Receptors are Expressed in Human Non-Small Cell Lung Carcinoma," *Life Sci*, 56(26):2385-92 (1995); Vilner BJ, et al., "Sigma-1 and Sigma-2 Receptors are Expressed in a Wide Variety of Human and Rodent Tumor Cell Lines.," *Cancer ReS*, 55(2):408-13 (1995); Everaert H, et al., "Sigma-Receptor Imaging by Means of I123-IDAB Scintigraphy: Clinical Application in Melanoma and Non-Small Cell Lung Cancer," *Anti-cancer Res*, 17(3B):1577-82 (1997); Glennon RA, et al., "Structural Features Important for Sigma 1 Receptor Binding," *J Med Chem*, 37(8):1214-9 (1994); John CS, et al., "Substituted Halogenated Arylsulfonamides: A New Class Of Sigma Receptor Binding Tumor Imaging Agents," *J Med Chem*, 41(14):2445-50 (1998); John CS, et al., "Synthesis and Characterization of [125I]-N-(N-benzylpiperidin-4-yl)-4- iodobenzamide, a New Sigma Receptor Radiopharmaceutical High-affinity Binding to MCF-7 Breast Tumor Cells," *J Med Chem*, 37(12):1737-9 (1994); Dence CS, John CS, Bowen WD, Welch MJ, "Synthesis and Evaluation of [18F] Labeled Benzamides: High Affinity Sigma Receptor Ligands for PET Imaging," *Nucl Med Biol*, 24(4):333-40 (1997); de Costa BR, et al., "Synthesis and Evaluation of Optically Pure [3H]-(+)-

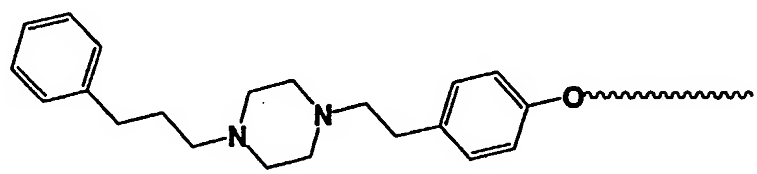
pentazocine, A Highly Potent and Selective Radioligand for Sigma Receptors," *FEBS Lett*, 251(1-2):53-8 (1989); Waterhouse RN, Mardon K, O'Brien JC, "Synthesis and Preliminary Evaluation of [123I]1-(4-cyanobenzyl)-4-[[(trans-iodopropen-2-yl)oxy]methyl]piperidin e: A Novel High Affinity Sigma Receptor Radioligand for SPECT," *Nucl Med Biol*, 24(1):45-5 (1997); John CS, et al., "Synthesis, In Vitro Pharmacologic Characterization, and Preclinical Evaluation of N-[2-(1'-piperidinyl)ethyl]-3-[125I]iodo-4-methoxybenzamide (P[125I]MBA) for Imaging Breast Cancer," *Nucl Med Biol*, 26(4):377-82 (1999); John CS, et al., "Synthesis, In Vitro Validation and In Vivo Pharmacokinetics of [125I]N-[2-(4-iodophenyl)ethyl]-N-methyl-2-(1-piperidinyl) ethylamine: A High-Affinity Ligand for Imaging Sigma Receptor Positive Tumors," *Nucl Med Biol*, 23(6):761-6 (1996); Huang Y., et al., "Synthesis and Quantitative Structure-activity Relationships of N-(1-benzylpiperidin-4-yl)phenylacetamides and Related Analogues as Potent and Selective Sigma1 Receptor Ligands," *J Med Chem*, 41(13):2361-70 (1998); Berardi F., et al., "N-[omega-(Tetralin-1-yl)alkyl] Derivatives of 3,3-Dimethylpiperidine are Highly Potent and Selective Sigma1 or Sigma2 Ligands," *J Med Chem*, 41(21):3940-7 (1998), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to sigma receptors.

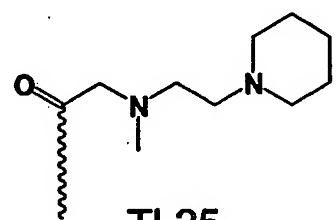
A large variety of lipophilic piperazines are known to bind with high affinity to sigma receptors. The following reference relates to this subject matter: Zhang Y. et al., "Characterization of Novel N,N'-disubstituted Piperazines as Sigma

Receptor Ligands," *J Med Chem*, 41(25):4950-7 (1998), the contents of which is incorporated herein by reference in its entirety.

In preferred embodiments (TL24 and TL25) the targeting ligands comprise the following structures:



TL24



TL25

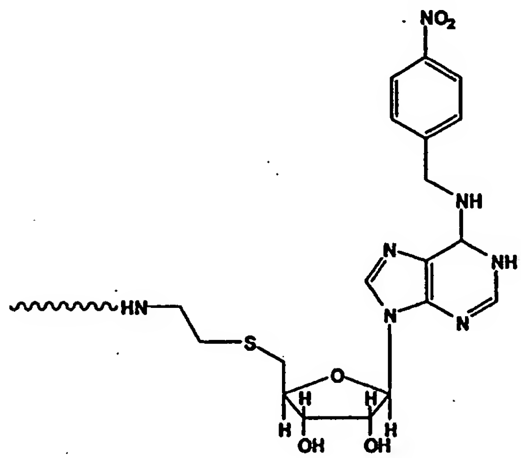
wherein the wavy line is the site of linker attachment to the remainder of the drug complex.

The following reference relates to this subject matter: John CS, et al., "99mTc-labeled Sigma-Receptor-Binding Complex: Synthesis, Characterization, and Specific Binding to Human Ductal Breast Carcinoma (T47D) Cells," *Bioconjug Chem*, 8(3):304-9 (1997), the contents of which is incorporated herein by reference in its entirety.

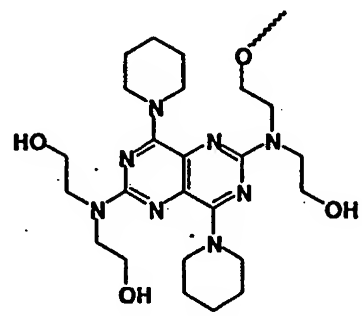
Nucleoside Transporter Targeting Ligands

Nucleoside Transporter (NT) catalyzes the equilibrative transport of nucleosides into cells. The transporter is markedly over-expressed in malignant cells following exposure to agents that interfere with the denovo synthesis of nucleosides derivatives. For example, human bladder cancer cells treated with an inhibitor, to a thymidylate synthase inhibitor displayed a 39 times increase in the amount of nucleoside transporter protein. Potent inhibitors of NT include the drugs dipyridamole and Dilazep. In addition, nitrobenzylthioadenosine analogs are potent inhibitors. The following references relate to this subject matter: Griffiths M., et al., "Cloning of a Human Nucleoside Transporter Implicated in the Cellular Uptake of Adenosine and Chemotherapeutic Drugs," *Nat Med,* 3(1):89-93 (1997); Pressacco J., et al., "Effects of Thymidylate Synthase Inhibition on Thymidine Kinase Activity and Nucleoside Transporter Expression," *Cancer Res,* 55(7):1505-8 (1995); Belt J.A., et al., "Nucleoside Transport in Normal and Neoplastic Cells," *Adv Enzyme Regul,* 33:235-52 (1993); Wiley J.S., et al., "A New Fluorescent Probe for the Equilibrative Inhibitor-Sensitive Nucleoside Transporter. 5'-S-(2-Aminoethyl)-N6-(4-Nitrobenzyl)-5'-Thioadenosine (SAENTA)-chi 2-Fluorescein," *Biochem J,* 273(Pt 3):667-72 (1991); Agbanyo F.R., et al., "5'-S-(2-Aminoethyl)-N6-(4-Nitrobenzyl)-5'-Thioadenosine (SAENTA), a Novel Ligand with High Affinity for Polypeptides Associated with Nucleoside Transport. Partial Purification of the Nitrobenzylthioinosine-Binding Protein of Pig Erythrocytes by Affinity Chromatography," *Biochem J,* 270(3):605-14 (1990); Baldwin S.A., et al., "Nucleoside Transporters: Molecular Biology and Implications for Therapeutic Development," *Mol Med Today,* 5:216-224 (1999), the contents of which are incorporated herein by reference in their entirety.

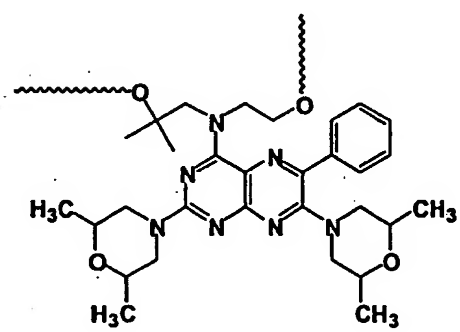
A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to nucleoside transporter proteins. In preferred embodiments (TL26, TKL27, TL28 and TL29)) the targeting ligands comprise the following structures:



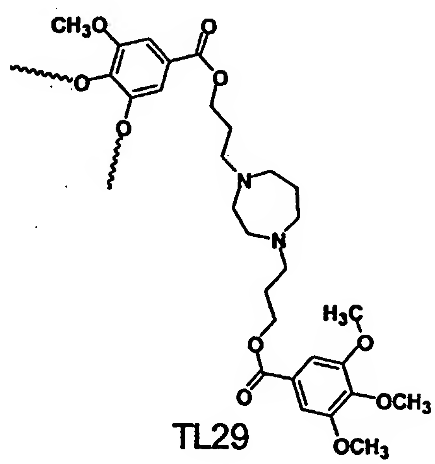
TL26



TL 27



TL28



TL29

wherein the wavy line is the site of linker attachment to the remainder of the drug ET or H.

wherein the wavy line is the site of linker attachment to the remainder of the drug ET or H.

Folate Receptor Targeted Ligands

The high affinity folate receptor (FR) is over-expressed in a number of malignancies including most ovarian and endometrial carcinomas and some breast, lung, colorectal, and renal cell cancers. Nonmucinous ovarian carcinomas have levels of FR that are increased 80 to 90 times over the levels present in normal ovaries. FR is also widely distributed in normal tissues with high levels in normal kidney, lung, thyroid, and choroidal plexus. Substantial efforts have been directed towards targeting folate receptors for diagnosis and therapy of FR+ malignancies. Monoclonal antibodies, conjugates of folic acid and radiolabelled groups, conjugates of folic acid and cytotoxic agents, and cancer vaccines have all been explored. A major barrier to success is the high concentration of FR in vital locations such as the kidney and choroid plexus. In rats radio-imaging studies have demonstrated intense accumulation in normal kidneys of FR targeted compounds. The following references relate to this subject matter: Susten SS, et al., "A Fluorescent Analogue of Methotrexate as a Probe for Folate Antagonist Molecular Receptors," *Biochem Pharmacol*, 33(12):1957-62 (1984); Holm J., et al., "A High-Affinity Folate Binding Protein in Proximal Tubule Cells of Human Kidney," *Kidney Int*, 41(1):50-5 (1992); Kranz DM, et al., "Conjugates of Folate and Anti-T-Cell-Receptor Antibodies Specifically Target Folate-Receptor-Positive Tumor Cells for Lysis," *Proc Natl Acad Sci USA*, 92(20):9057-61 (1995); Westerhof GR, et al., "Carrier- and

Receptor-Mediated Transport of Folate Antagonists Targeting Folate-Dependent Enzymes: Correlates of Molecular-Structure and Biological Activity," *Mol Pharmacol*, 48(3):459-71 (1995); Wang S., et al., "Design and Synthesis of [111In]DTPA-Folate for use as a Tumor-Targeted Radiopharmaceutical," *Bioconjug Chem*, 8(5):673-9 (1997); Rothberg KG, et al., "The Glycophospholipid-Linked Folate Receptor Internalizes Folate Without Entering the Clathrin-Coated Pit Endocytic Pathway," *J Cell Biol*, 110(3):637-49 (1990); Fan J., et al., "Novel Substrate Analogs Delineate an Endocytotic Mechanism for Uptake of Folate via the High-Affinity, Glycosylphosphatidylinositol-Linked Transport Protein in L1210 Mouse Leukemia Cells," *Oncol Res*, 7(10-11):511-6 (1995); Mantovani LT, et al., "Folate Binding Protein Distribution in Normal Tissues and Biological Fluids from Ovarian Carcinoma Patients as Detected by the Monoclonal Antibodies MOv18 and MOv19," *Eur J Cancer*, 30A(3):363-9 (1994); Leamon CP; Low PS, "Membrane Folate-Binding Proteins are Responsible for Folate-Protein Conjugate Endocytosis into Cultured Cells," *Biochem J*, 291 (Pt 3):855-60 (1993); Holm J, et al., "Folate Receptor of Human Mammary Adenocarcinoma," *APMIS*, 102(6):413-9 (1994); Holm J., et al., "Folate Receptors in Malignant and Benign Tissues of Human Female Genital Tract," *Biosci Rep*, 17(4):415-27 (1997); Wang S., et al., "Synthesis, Purification, and Tumor Cell Uptake of 67Ga-deferoxamine– Folate, a Potential Radiopharmaceutical for Tumor Imaging," *Bioconjug Chem*, 7(1):56-62 (1996); Mathias CJ, et al., "Tumor-Selective Radiopharmaceutical Targeting via Receptor-Mediated Endocytosis of Gallium-67-Deferoxamine-Folate," *J Nucl Med*, 37(6):1003-8 (1996); Ladino CA, et al., "Folate-Cantansinoids: Target-Selective Drugs of Low Molecular Weight," *Int J Cancer*, 73(6):859-64 (1997); Li

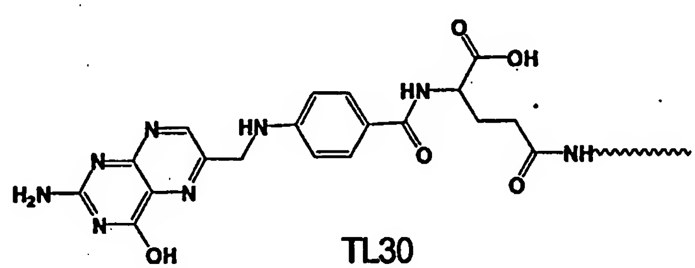
S; Deshmukh HM; Huang L., "Folate-Mediated Targeting of Antisense Oligodeoxynucleotides to Ovarian Cancer Cells," *Pharm Res*, 15(10):1540-5 (1998); Reddy JA, Low PS, "Folate-Mediated Targeting of Therapeutic and Imaging Agents to Cancers," *Crit Rev Ther Drug Carrier Syst*, 15(6):587-627 (1998); Leamon CP; Low PS, "Membrane Folate-Binding Proteins are Responsible for Folate-Protein Conjugate Endocytosis into Cultured Cells," *Biochem J*, 291 (Pt 3):855-60 (1993); Leamon CP; Low PS, "Delivery of Macromolecules into Living Cells: a Method that Exploits Folate Receptor Endocytosis," *Proc Natl Acad Sci USA*, 88(13):5572-6 (1991); Ginobbi P., et al., "Folic Acid-Polylysine Carrier Improves Efficacy of c-myc Antisense Oligodeoxynucleotides on Human Melanoma (M14) Cells," *Anti-cancer Res*, 17(1A):29-35 (1997); Holm J., et al., "High-Affinity Folate Receptor in Human Ovary, Serous Ovarian Adenocarcinoma, and Ascites: Radioligand Binding Mechanism, Molecular Size, Ionic Properties, Hydrophobic Domain, and Immunoreactivity," *Arch Biochem Biophys*, 366(2):183-91 (1999); Reddy JA; Low PS, "Folate-Mediated Targeting of Therapeutic and Imaging Agents to Cancers [In Process Citation]," *Crit Rev Ther Drug Carrier Syst*, 15(6):587-627 (1998); Li PY, et al., "Local Concentration of Folate Binding Protein GP38 in Sections of Human Ovarian Carcinoma by In Vitro Quantitative Autoradiography," *J Nucl Med*, 37(4):665-72 (1996); Toffoli G., et al., "Over-expression of Folate Binding Protein in Ovarian Cancers," *Int J Cancer*, 74(2):193-8 (1997); Birn H; Selhub J; Christensen EI, "Internalization and Intracellular Transport of Folate-Binding Protein in Rat Kidney Proximal Tubule," *Am J Physiol*, 264(2 Pt 1):C302-10 (1993); Abraham A., et al., "Folate Analogues. 33. Synthesis of Folate and Antifolate Poly-Gamma- Glutamates by

[(9-fluorenylmethoxy)oxy]carbonyl Chemistry and Biological Evaluation of Certain Methotrexate Polyglutamate polylysine Conjugates as Inhibitors of the Growth of H35 Hepatoma Cells," *J Med Chem*, 33(2):711-7 (1990); Gabizon A, et al., "Targeting Folate Receptor with Folate Linked to Extremities of Poly(Ethylene Glycol)-Grafted Liposomes: In Vitro Studies," *Bioconjug Chem*, 10(2):289-98 (1999); Ladino CA, et al., "Folate-Cantansinoids: Target-Selective Drugs of Low Molecular Weight," *Int J Cancer*, 73(6):859-64 (1997); Leamon CP, Low PS, et al., "Selective Targeting of Malignant Cells with Cytotoxin-Folate Conjugates," *J. Drug Target*, 2(2):101-1 (1994); Konda S.D., et al., "Development of a Tumor-Targeting MR Contrast Agent using the High-Affinity Folate Receptor: Work In Progress," *Invest Radiol*, 35(1):50-7 (2000); Toffoli G., et al., "Expression of Folate Binding Protein as a Prognostic Factor for Response to Platinum-Containing Chemotherapy and Survival in Human Ovarian Cancer," *Int J Cancer*, 79(2):121-6 (1998); Lu J.Y., et al., "Folate-Targeted Enzyme Prodrug Cancer Therapy Utilizing Penicillin-V Amidase and a Doxorubicin Prodrug," *J Drug Target*, 7(1):43-53 (1999); Sudimack J; Lee R.J., "Targeted Drug Delivery via the Folate Receptor," *Adv Drug Deliv Rev*, 41(2):147-162 (2000); Peoples G.E., et al., "Vaccine Implications of Folate Binding Protein, a Novel Cytotoxic T Lymphocyte-Recognized Antigen System in Epithelial Cancers," *Clin Cancer Res*, 5(12):4214-23 (1999), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to the high affinity folate receptor. The multifactorial properties of multifunctional drug delivery vehicles

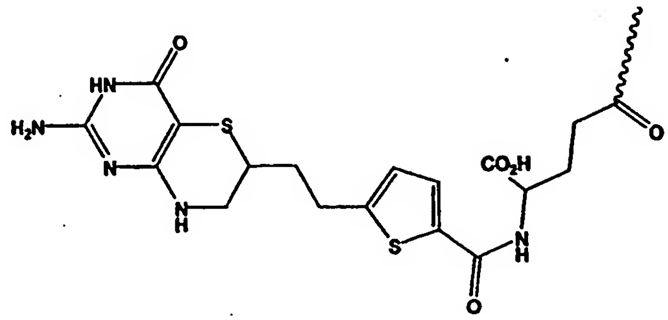
can allow the FR to be exploited as a tumor target without damage to FR+ non-tumor tissues. For example, a drug targeted to ovarian cancer with targeting ligands for FR and MMP-7 and fatty acid synthase can have increased selectivity for ovarian cancer. Adult kidneys lack or have very low levels of both fatty acid synthase and MMP-7.

In preferred embodiments (TL30)) the targeting ligands comprise the following structures:



TL30

Another preferred embodiment (TL31) shown below is based upon the ability of bicyclic 5-thiapyrimidinones to bind with subpicomolar affinity to the FR. The following references relate to this subject matter: Varney M.D., et al., "Protein Structure-Based Design, Synthesis, and Biological Evaluation of 5-Thia-2,6-diamino-4(*3H*)-oxopyrimidines: Potent Inhibitors of Glycinamide Ribonucleotide Transformylase with Potent Cell Growth Inhibition," *J Med Chem*, 40:2502-2524 (1997), the contents of which are incorporated herein by reference in their entirety.



TL31

wherein the wavy line is the site of linker attachment to the remainder of the drug complex.

Somatostatin Receptor Targeted Ligands

Somatostatin receptors (SSR) are expressed at high levels in a variety of human malignancies including: breast, prostate, neuroblastoma, medullabalstoma, pancreatic, ovarian, gastrinoma, thyroid, melanoma, renal, lymphoma, glioma, colorectal, small cell lung cancer, and most neuroendocrine tumors. The over-expression of somatostatin receptors on malignant cells has been utilized for both diagnostic and therapeutic purposes. A large variety of radiolabelled SSR analogs have been developed. In addition, conjugates of potent cytotoxic agents have been coupled to SSR binging groups as potential antineoplastic drugs. In addition, a large number of analogs, which bind to SSR, have been investigated as anti-cancer therapies. The potential of SSR targeted therapies is currently limited by the fact that the receptor is not uniquely specific for cancer cells.

Somatostatin receptors are present in important normal tissues including the brain, pituitary, adrenal glands, pancreas, gastrointestinal tract, and kidney. The

following references relate to this subject matter: Forssell-Aronsson E.B., et al.," 111In-DTPA-D-Phe1-octreotide Binding and Somatostatin Receptor Subtypes in Thyroid Tumors," *J Nucl Med,* 41(4):636-42 (2000); Sulkowski U., et al., "A Phase II Study of High-Dose Octreotide in Patients with Unresectable Pancreatic Carcinoma," *Eur J Cancer,* 35(13):1805-8 (1999); Reubi J.C., et al., "A Selective Analog for the Somatostatin sst1-Receptor Subtype Expressed by Human Tumors," *Eur J Pharmacol,* 345(1):103-10 (1998); O'Byrne K.J. et al., "Phase II Study of RC-160 (Vapreotide), an Octapeptide Analogue of Somatostatin, in the Treatment of Metastatic Breast Cancer," *Br J Cancer,* 79(9-10):1413-8 (1999); Bajc M., et al., "Dynamic Indium-111-Pentetreotide Scintigraphy in Breast Cancer," *J Nucl Med,* 37(4):622-6 (1996); Schally A.V.; Nagy A., "Cancer Chemotherapy Based on Targeting of Cytotoxic Peptide Conjugates to their Receptors on Tumors," *Eur J Endocrinol,* 141(1):1-14 (1999Nagy A., et al., "Synthesis and Biological Evaluation of Cytotoxic Analogs of Somatostatin Containing Doxorubicin or its Intensely Potent Derivative, 2-Pyrrolinodoxorubicin," *Proc Natl Acad Sci USA,* 95(4):1794-9 (1998); Sinisi A.A., et al., "Different Expression Patterns of Somatostatin Receptor Subtypes in Cultured Epithelial Cells from Human Normal Prostate and Prostate Cancer," *J Clin Endocrinol Metab,* 82(8):2566-9 (1997); Albert R., et al., "Direct Synthesis of [DOTA-DPhe1]-octreotide and [DOTA-DPhe1,Tyr3]-octreotide (SMT487): Two Conjugates for Systemic Delivery of Radiotherapeutical Nuclides to Somatostatin Receptor Positive Tumors in Man,." *Bioorg Med Chem Lett,* 8(10):1207-10 (1998); Reubi Plonowski A., et al., "Inhibition of PC-3 Human Androgen-Independent Prostate Cancer and its Metastases by Cytotoxic Somatostatin Analogue AN-238," *Cancer Res,* 59(8):1947-53 (1999); Papotti M.,

et al., "Correlative Immunohistochemical and Reverse Transcriptase Polymerase Chain Reaction Analysis of Somatostatin Receptor Type 2 in Neuroendocrine Tumors of the Lung," *Diagn Mol Pathol*, 9(1):47-57 (2000); Moertel C.L., et al., "Expression of Somatostatin Receptors in Childhood Neuroblastoma," *Am J Clin Pathol*, 102(6):752-6 (1994); Lewis J.S., et al., "In vitro and in Vivo Evaluation of 64Cu-TETA-Tyr3-Octreotate. a New Somatostatin Analog with Improved Target Tissue Uptake," *Nucl Med Biol*, 26(3):267-73 (1999);; Plonowski A., et al., "Inhibition of PC-3 Human Androgen-Independent Prostate Cancer and its Metastases by Cytotoxic Somatostatin Analogue AN-238," *Cancer Res*, 59(8):1947-53 (1999); Koppan M., et al., "Targeted Cytotoxic Analogue of Somatostatin AN-238 Inhibits Growth of Androgen-Independent Dunning R-3327-AT-1 Prostate Cancer in Rats at Nontoxic Doses," *Cancer Res*, 58(18):4132-7 (1998); Nilsson S., et al., "Metastatic Hormone-Refractory Prostatic Adenocarcinoma Expresses Somatostatin Receptors and is Visualized in Vivo by [111In]-labeled DTPA-D-[Phe1]-octreotide Scintigraphy," *Cancer Res*, 55(23 Suppl):5805s-5810s (1995); Briganti V., et al., "Imaging of Somatostatin Receptors by Indium-111-Pentetreotide Correlates with Quantitative Determination of Somatostatin Receptor Type 2 Gene Expression in Neuroblastoma Tumors," *Clin Cancer Res*, 3(12 Pt 1):2385-91 (1997); Thakur M.L., et al., "Radiolabeled Somatostatin Analogs in Prostate Cancer," *Nucl Med Biol*, 24(1):105-13 (1997); Reubi J.C.; Kvols L., "Somatostatin Receptors in Human Renal Cell Carcinomas," *Cancer Res*, 52(21):6074-8 (1992); Vitale G., et al., "Slow Release Lanreotide in Combination with Interferon-Alpha2b in the Treatment of Symptomatic Advanced Medullary Thyroid Carcinoma," *J Clin Endocrinol Metab*, 85(3):983-8 (2000); Reisinger I., et al., "Somatostatin

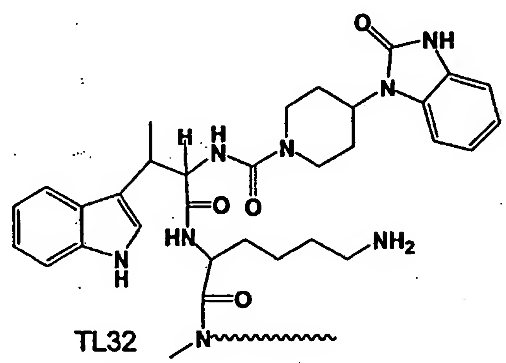
Receptor Scintigraphy in Small-Cell Lung Cancer: Results of a Multicenter Study," *J Nucl Med*, 39(2):224-7 (1998); Albini A., et al., "Somatostatin Controls Kaposi's Sarcoma Tumor Growth through Inhibition of Angiogenesis," *FASEB J*, 13:647-655 (1999); Reubi J.C., et al., "Expression and Localization of Somatostatin Receptor SSTR1, SSTR2, and SSTR3 Messenger RNas in Primary Human Tumors using in Situ Hybridization," *Cancer Res*, 54(13):3455-9 (1994); Vuaroqueaux V., et al., "No Loss of sst Receptors Gene Expression in Advanced Stages of Colorectal Cancer," *Eur J Endocrinol*, 140(4):362-6 (1999); Kahan Z., et al., "Inhibition of Growth of MX-1, MCF-7-MIII and MDA-MB-231 Human Breast Cancer Xenografts after Administration of a Targeted Cytotoxic Analog of Somatostatin, AN-238," *Int J Cancer*, 82(4):592-8 (1999); Krenning E.P., et al., "The Role of Radioactive Somatostatin and its Analogues in the Control of Tumor Growth," *Recent Results Cancer Res*, 153:1-13 (2000); Kath R.; Hoffken K. "The "Yttrium-90 DOTATOC: First Clinical Results," *Eur J Nucl Med*, 26(11):1439-47 (1999); Friedberg et al., "Octreotide Scanning in Metastatic Sarcoma," *Cancer*, 86:1621-7 (1999); Froidevaux S., et al., "Receptor Targeting for Tumor Localisation and Therapy with Radiopeptides," *Curr Med Chem*, 7(9):971-994 (2000); Freidinger R.M., "Nonpeptidic Ligands for Peptide and Protein Receptors," *Curr Opin Chem Biol*, 3(4):395-406 (1999), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to somatostatin receptors.

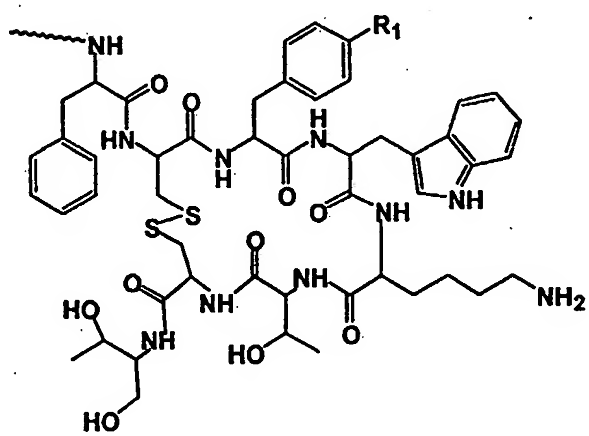
A preferred embodiment is based on the ability of a class of small peptidomimetics that bind to type 2 SSR with picomolar potency. The following

references relate to this subject matter: Yang L., et al., "Synthesis and Biological Activities of Potent Peptidomimetics Selective for Somatostatin Receptor Subtype 2," *Proc Natl Acad Sci USA*, 95(18):10836-41 (1998), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (TL32 and TL33) the targeting ligands comprise the following structures:



TL32



TL 33

wherein the wavy line is the site of linker attachment to the remainder of the drug complex.

Other preferred embodiments are based on the ability of somatostatin analogs substituted on the amino terminus with chelating agents to retain the ability to bind to SSR. The following references relate to this subject matter: Lewis J.S., et al., "Comparison of Four ^{64}Cu-Labeled Somatostatin Analogues in Vitro and in a Tumor-Bearing Rat Model: Evaluation of New Derivatives for Positron Emission Tomography Imaging and Targeted Radiotherapy," *J Med Chem,* 42(8):1341-1347 (1999), the contents of which are incorporated herein by reference in their entirety.

Wherein for TL33 the wavy line is the site of linker attachment to the remainder of the drug complex and R_1 is H, or OH, and the terminal phenylalanine and the tryptophan have the D- configuration and the remainder of the amino acid residues have the L-configuration.

Gastrin Releasing Peptide Receptor Targeting Ligands

Gastrin releasing peptide receptors (GRPR) are over-expressed in a variety of malignancies including: lung, breast, prostate, colorectal, gastric, and melanoma. Gastrin releasing peptide is produced in small cell lung carcinoma in an autocrine manner and stimulates cell growth by binding to GRPR. A large variety of radiolabelled GRPR analogs have been developed. Conjugates of potent cytotoxic agents have been coupled to GRPR binding groups as potential antineoplastic drugs. In addition a large number of analogs that bind to GRPR have been investigated as anti-cancer therapies. However, GRPR is not specific to malignant cells, which currently limits its utility as an anti-cancer target.

Normal tissues that express significant amounts of GRPR include the gastric antrum, breast, ovarian, pancreas, brain, and skin. The following references relate to this subject matter: Karra S. R., et al., "^{99m}Tc-Labeling and in Vivo Studies of a Bombesin Analogue with a Novel Water-Soluble Dithiadiphosphine-Based Bifunctional Chelating Agent," *Bioconjugate Chem*, 10(2):254–260 (1999); Carroll R.E., et al., "Aberrant Expression of Gastrin-Releasing Peptide and its Receptor by Well-Differentiated Colon Cancers in Humans," *AJP-Gastrointestinal and Liver Physiology*, 276 (3):G655-G665 (1999); Wang Q.J., et al., "Bombesin Can Stimulate Proliferation of Human Pancreatic Cancer Cells through an Autocrine Pathway," *Int J Cancer*, 68(4):528-34 (1996); Carroll R.E., et al., "Characterization of Gastrin-Releasing Peptide Receptors Aberrantly Expressed by Non-Antral Gastric Adenocarcinomas," *Peptides*, 20(2):229-37 (1999); Chave H.S., et al., "Bombesin Family Receptor and Ligand Gene Expression in Human Colorectal Cancer and Normal Mucosa," *Br J Cancer*, 82(1):124-30 (2000); Azay J., et al., "Comparative Study of in Vitro and in Vivo Activities of Bombesin Pseudopeptide Analogs Modified on the C-Terminal Dipeptide Fragment," *Peptides*, 19(1):57-63 (1998); Nagy A., et al., "Design, Synthesis, and in Vitro Evaluation of Cytotoxic Analogs of Bombesin-Like Peptides Containing Doxorubicin or its Intensely Potent Derivative, 2-Pyrrolinodoxorubicin," *Proc Natl Acad Sci U S A*, 94(2):652-6 (1997); Baidoo K.E., et al., "Design, Synthesis, and Initial Evaluation of High-Affinity Technetium Bombesin Analogues," *Bioconjugate Chem.*, 9(2):218 -225, (1998); Breeman W.A., et al., "Evaluation of Radiolabelled Bombesin Analogues for Receptor-Targeted Scintigraphy and Radiotherapy," *Int J Cancer*, 81(4):658-65 (1999); Tang C., et al. "Expression of Receptors for Gut Peptides in Human Pancreatic

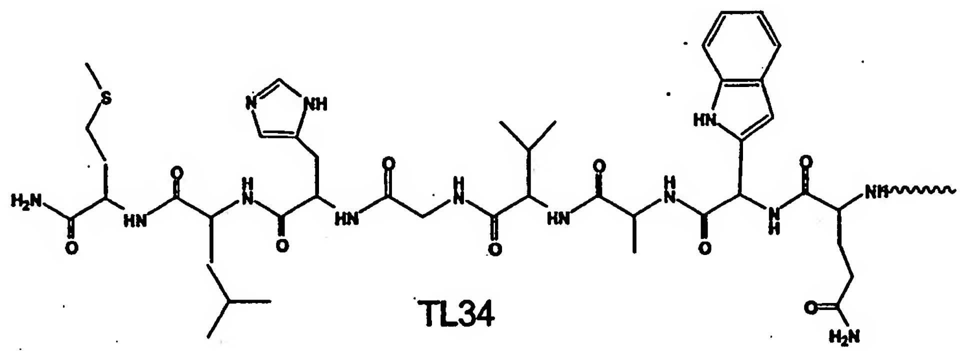
Adenocarcinoma and Tumour-Free Pancreas," *Br J Cancer*, 75(10):1467-73 (1997); Sainz E., et al., "Four Amino Acid Residues are Critical for High Affinity Binding of Neuromedin B to the Neuromedin B Receptor," *J Biol Chem*, 273(26):15927-15932 (1998); Kiaris H., et al., "Targeted Cytotoxic Analogue of Bombesin/Gastrin-Releasing Peptide Inhibits the Growth of H-69 Human Small-Cell Lung Carcinoma in Nude Mice," *Br J Cancer*, 81(6):966-71 (1999); Gugger M.; Reubi J.C., "Gastrin-Releasing Peptide Receptors in Non-Neoplastic and Neoplastic Human Breast," *American Journal of Pathology*, 155:2067-2076 (1999); Markwalder R.; Reubi J.C., "Gastrin-Releasing Peptide Receptors in the Human Prostate: Relation to Neoplastic Transformation," *Cancer Res*, 59(5):1152-9 (1999); Sun B., et al., "The Presence of Receptors for Bombesin/GRP and Mrna for Three Receptor Subtypes in Human Ovarian Epithelial Cancers," *Regul Pept*, 90(1-3):77-84 (2000); Sun B., et al., "Presence of Receptors for Bombesin/Gastrin-Releasing Peptide and Mrna for Three Receptor Subtypes in Human Prostate Cancers," *Prostate*, 42(4):295-303 (2000); Pradhan T.K., et al. "Identification of a Unique Ligand which has High Affinity for all Four Bombesin Receptor Subtypes," *Eur J Pharmacol*, 343(2-3):275-87 (1998); Pansky A., et al., "Identification of Functional GRP-Preferring Bombesin Receptors on Human Melanoma Cells," *Eur J Clin Invest*, 27(1):69-76 (1997); Bartholdi M.F., et al., "In Situ Hybridization for Gastrin-Releasing Peptide Receptor (GRP Receptor) Expression in Prostatic Carcinoma," *Int J Cancer*, 79(1):82-90 (1998); Jungwirth A., et al.," Inhibition of Growth of Androgen-Independent DU-145 Prostate Cancer in Vivo by Luteinising Hormone-Releasing Hormone Antagonist Cetrorelix and Bombesin Antagonists RC-3940-II and RC-3950-II," *Eur J Cancer*, 33(7):1141-8 (1997); Kahan Z., et al., "Inhibition of

Growth of MDA-MB-468 Estrogen-Independent Human Breast Carcinoma by Bombesin/Gastrin-Releasing Peptide Antagonists RC-3095 and RC-3940-II," *Cancer*, 88(6):1384-92 (2000); Ferris H.A., et al., "Location and Characterization of the Human GRP Receptor Expressed by Gastrointestinal Epithelial Cells," *Peptides*, 18(5):663-72 (1997); Toi-Scott M., et al., "Clinical Correlates of Bombesin-Like Peptide Receptor Subtype Expression in Human Lung Cancer Cells," *Lung Cancer*, 15(3):341-54 (1996); Jungwirth A., et al., "Luteinizing Hormone-Releasing Hormone Antagonist Cetrorelix (SB-75) and Bombesin Antagonist RC-3940-II Inhibit the Growth of Androgen-Independent PC-3 Prostate Cancer in Nude Mice," *Prostate*, 32(3):164-72 (1997); Safavy A., et al., "Paclitaxel Derivatives for Targeted Therapy of Cancer: Toward the Development of Smart Taxanes," *J. Med. Chem.*, 42(23):4919 -4924 (1999); Katsuno T., et al., "Pharmacology and Cell Biology of the Bombesin Receptor Subtype 4 (BB_4-R)," *Biochemistry*, 38(22):7307–7320 (1999); Breeman W.A., et al., "Pre-clinical Evaluation of [(111)In-DTPA-Pro(1), Tyr(4)]Bombesin, a New Radioligand for Bombesin-Receptor Scintigraphy," *Int J Cancer*, 83(5):657-63 (1999); Halmos G.; Schally A.V., "Reduction in Receptors for Bombesin and Epidermal Growth Factor in Xenografts of Human Small-Cell Lung Cancer after Treatment with Bombesin Antagonist RC-3095," *Proc. Natl. Acad. Sci. USA*, 94:956-960 (1997); Miyazaki M., et al., "Inhibition of Growth of MDA-MB-231 Human Breast Cancer Xenografts in Nude Mice by Bombesin/Gastrin-Releasing Peptide (GRP) Antagonists RC-3940-II and RC-3095," *Eur J Cancer*, 34(5):710-7 (1998); Staniek V., et al., "Expression of Gastrin-Releasing Peptide Receptor in Human Skin," *Acta Derm Venereol*, 76(4):282-6 (1996); Llinares M., et al., "Syntheses and Biological Activities of Potent Bombesin Receptor Antagonists,"

J Pept Res, 53(3):275-83 (1999); Cristau M., et al., "Synthesis and Biological Evaluation of Bombesin Constrained Analogues," *Med Chem ASAP Article*, 10:1021 (Can 12, 2000); Carrithers M.D.; Lerner M.R., "Synthesis and Characterization of Bivalent Peptide Ligands Targeted to G-Protein-Coupled Receptors," *Chem Biol*, 3(7):537-42 (1996); Safavy A., et al., "Synthesis of Bombesin Analogues for Radiolabeling with Rhenium-188," *Cancer*, 80(12 Suppl):2354-9 (1997); Slice L.W., et al., "Visualization of Internalization and Recycling of the Gastrin Releasing Peptide Receptor-Green Fluorescent Protein Chimera Expressed in Epithelial Cells," *Receptors Channels*, 6(3):201-12 (1998), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to GRPR. A preferred embodiment is based upon the ability of a bombesin analog with a chelating agent coupled to the amino terminus to bind with high affinity to GRPR. The following references relate to this subject matter: Karra S. R., et al., "^{99m}Tc-Labeling and in Vivo Studies of a Bombesin Analogue with a Novel Water-Soluble Dithiadiphosphine-Based Bifunctional Chelating Agent," *Bioconjugate Chem*, 10(2):254–260 (1999), the contents of which are incorporated herein by reference in their entirety.

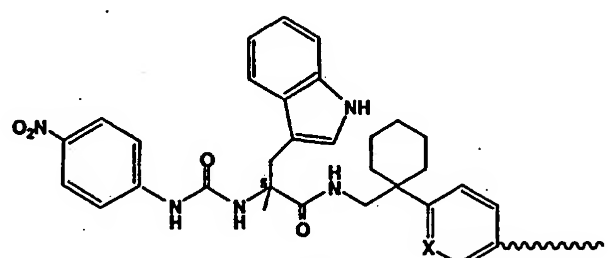
In preferred embodiments (TL34 and TL35) the targeting ligands comprise the following structures:



TL34

wherein the wavy line is the site of linker attachment to the remainder of the drug.

Another preferred embodiment is based on the ability of the nonpeptide gastrin releasing receptor antagonist to bind with high affinity to GRPR.



TL35

wherein the wavy line is the site of linker attachment to the remainder of the drug, and X is C, or N. The following references relate to this subject matter: Ashwood V., et al., "PD 176252–The First High Affinity Non-Peptide Gastrin-Releasing Peptide (BB2) Receptor Antagonist," *Bioorg Med Chem Lett*, 8(18):2589-94 (1998), the contents of which are incorporated herein by reference in their entirety.

Melanocyte Stimulating Hormone Receptor Targeting Ligands

Melanocyte Stimulating Hormone Receptors (MSHR) bind melanocyte stimulating hormone and related peptide factors with high affinity. The MSH receptors are localized to melanocytes, keratinocytes, monocytes, macrophages, and the dermal microvasculature. The consistent expression of MSHR in malignant melanoma has stimulated efforts to employ the receptor for diagnostic imaging and chemotherapy targeting. A large number of potent analogs are known which bind with high affinity to this receptor. Compounds with multiple copies of MSHR binding ligands have been prepared for diagnostic and potential therapeutic use. MSHR are present at low density with approximately 2,000 to 10,000 receptor molecules/cell. The low receptor density necessitates the delivery of an extremely potent toxin that is cytotoxic at sub-nanomolar concentrations to kill the MSHR positive tumor cells. To achieve selective toxicity the concentration of targeted drug required to saturate the receptors on the tumor cells must be even lower. Also, melanoma cells secrete melanocyte stimulating hormone, which can act as a competitive inhibitor to MSHR targeted drugs. Accordingly, targeting affinity in the sub-picomolar range is required, which markedly exceeds the high binding affinity of currently known MSHR ligands. Even given a ligand with the requisite affinity the problem of toxicity to non-tumor MSHR positive keratinocytes and dermal blood vessels remains. Both of these problems can be solved with the present invention by utilizing a multifunctional drug delivery vehicle that incorporates a MSHR binding ligand a second ligand that binds with high affinity to a second target present at high concentrations on melanoma cells, but absent or present at low levels on

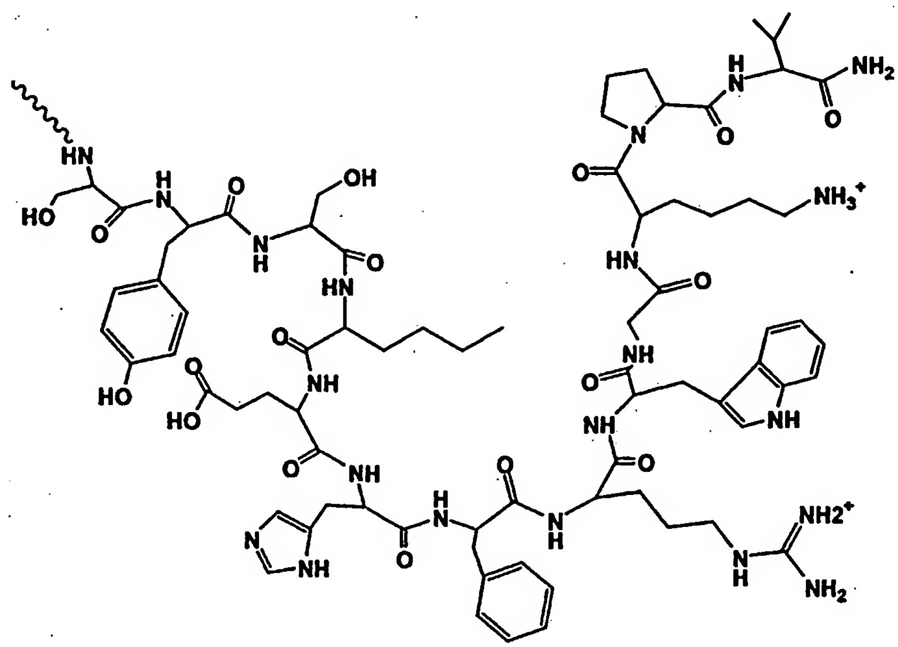
keratinocytes and dermal microvasculature. The following references relate to this subject matter: Tsatmali M., et al., "ACTH1-17 is a More Potent Agonist at the Human MC1 Receptor than Alpha-MSH," *Cell Mol Biol (Noisy-le-grand)*, 45(7):1029-34 (1999); Hruby V.J., et al., "Cyclic Lactam Alpha-Melanotropin Analogues of Ac-Nle4-cyclo[Asp5, D-Phe7,Lys10] Alpha-Melanocyte-Stimulating Hormone-(4-10)-NH2 with Bulky Aromatic Amino Acids at Position 7 Show High Antagonist Potency and Selectivity at Specific Melanocortin Receptors," *J Med Chem*, 38(18):3454-61 (1995); Funasaka Y., et al., 'Expression of Proopiomelanocortin, Corticotropin-Releasing Hormone (CRH), and CRH Receptor in Melanoma Cells, Nevus Cells, and Normal Human Melanocytes," *J Investig Dermatol Symp Proc*, 4(2):105-9 (1999); Vaidyanathan G.; Zalutsky M.R., "Fluorine-18-labeled [Nle4,D-Phe7]-alpha-MSH, an Alpha-Melanocyte Stimulating Hormone Analogue," *Nucl Med Biol*, 24(2):171-8 (1997); Jiang J., et al., "Human Epidermal Melanocyte and Keratinocyte Melanotropin Receptors: Visualization by Melanotropic Peptide Conjugated Macrospheres (Polyamide Beads)," *Exp Dermatol*, 6(1):6-12 (1997); Hartmeyer M., et al., "Human Dermal Microvascular Endothelial Cells Express the Melanocortin Receptor Type 1 and Produce Increased Levels of IL-8 Upon Stimulation with Alpha-Melanocyte-Stimulating Hormone," *J Immunol*, 159(4):1930-7 (1997); Loir B., et al., "Immunoreactive Alpha-Melanotropin as an Autocrine Effector in Human Melanoma Cells," *Eur J Biochem*, 244(3):923-30 (1997); Loir B., et al., "Expression of the MC1 Receptor Gene in Normal and Malignant Human Melanocytes. A Semiquantitative RT-PCR Study," *Cell Mol Biol (Noisy-le-grand)*, 45(7):1083-92 (1999); Rajora N., et al., "Alpha-MSH Production, Receptors, and Influence on Neopterin in a Human Monocyte/Macrophage Cell Line," *J Leukoc*

biol, 59(2):248-53 (1996); Sharma S.D., et al., "Melanotropic Peptide-Conjugated Beads for Microscopic Visualization and Characterization of Melanoma Melanotropin Receptors," *Proc Natl Acad Sci USA,* 93:13715-13720 (1996); Jiang J., et al., "Melanotropic Peptide Receptors: Membrane Markers of Human Melanoma Cells," *Exp Dermatol,* 5(6):325-33 (1996); Ghanem G.E., et al., "Human Melanoma Targeting with Alpha-MSH-Melphalan Conjugate," *Melanoma Res,* 1(2):105-14 (1991); Hadley M.E., et al., "[Nle4, D-Phe7]-alpha-MSH: A Superpotent Melanotropin that "Irreversibly" Activates Melanoma Tyrosinase," *Endocr Res,* 11(3-4):157-70 (1985); Sharma S.D. et al., "Multivalent Melanotropic Peptide and Fluorescent Macromolecular Conjugates: New Reagents for Characterization of Melanotropin Receptors," *Bioconjug Chem,* 5(6):591-601 (1994); Giblin M.F., et al., "Design and Characterization of α-Melanotropin Peptide Analogs Cyclized through Rhenium and Technetium Metal Coordination," *PNAS Online,* 95(22):12814-12818 (1998); O'Hare K.B, et al., "Polymeric Drug-Carriers Containing Doxorubicin and Melanocyte-Stimulating Hormone: In Vitro and in Vivo Evaluation against Murine Melanoma," *J Drug Target,* 1(3):217-29 (1993); Bagutti C., et al., "[111In]-DTPA-labeled Analogues of Alpha-Melanocyte-Stimulating Hormone for Melanoma Targeting: Receptor Binding in Vitro and in Vivo," *Int J Cancer,* 58(5):749-55 (1994); Morandini R., et al., "Receptor-Mediated Cytotoxicity of Alpha-MSH Fragments Containing Melphalan in a Human Melanoma Cell Line," *Int J Cancer,* 56(1):129-33 (1994); Ghanem G.E., et al., "Evidence for alpha-Melanocyte-Stimulating Hormone (Alpha-MSH) Receptors on Human Malignant Melanoma Cells," *Int J Cancer,* 41(2):248-55 (1988); Bednarek M.A., et al., "Structure-Function Studies on the Cyclic Peptide MT-II, Lactam Derivative of Alpha-Melanotropin,"

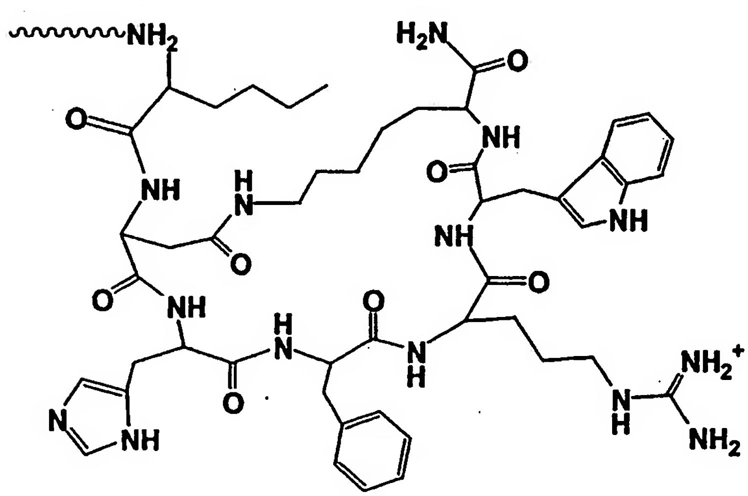
Peptides, 20(3):401-9 (1999); Chaturvedi D.N., et al., "Synthesis and Biological Evaluation of the Superagonist [N Alpha-Chlorotriazinylaminofluorescein-Ser1,Nle4,D-Phe7]-apha-MSH," *J Pharm Sci,* 74(3):237-40 (1985); Giblin M.F., et al., "Synthesis and Characterization of Rhenium-Complexed -Melanotropin Analogs," *Bioconjugate Chem,* 8(3):347-353 (1997); Brandenburger Y., et al., "Synthesis and Receptor Binding Analysis of Thirteen Oligomeric Alpha-MSH Analogs," *J Recept Signal Transduct Res,* 19(1-4):467-80 (1999); Erskine-Grout M.E, et al., "Melanocortin Probes for the Melanoma MC1 Receptor: Synthesis, Receptor Binding and Biological Activity," *Melanoma Res,* 6(2):89-94 (1996); Haskell-Luevano C., et al., "Truncation Studies of Alpha-Melanotropin Peptides Identify Tripeptide Analogues Exhibiting Prolonged Agonist Bioactivity," *Peptides,* 17(6):995-1002 (1996), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to MSHR. Preferred embodiments are based on some melanotropin analogs, which possess extremely high receptor affinity. It is known that the amino terminus can be substituted without impairing receptor binding. The following references relate to this subject matter: Haskell-Luevano C., et al., "Characterizations of the Unusual Dissociation Properties of Melanotropin Peptides from the Melanocortin Receptor, hMC1R," *J Med Chem,* 39:432-435 (1996), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (TL36 and TL37) the targeting ligands comprise the following structures:



TL36



TL37

wherein the wavy line is the site of linker attachement to the remainder of the drug complex. The following references relate to this subject matter: Haskell-Luevano C., et al., "Biological and Conformational Examination of Stereochemical Modifications Using the Template Melanotropin Peptide, Ac-Nle-c[Asp-His-Phe-Arg-Trp-Ala-Lys]-NH_2, on Human Melanocortin Receptors," *J Med Chem,* 40:1738-1748 (1997), the contents of which are incorporated herein by reference in their entirety.

Gastrin/Cholecystokinin Type B Receptor Targeting Ligands

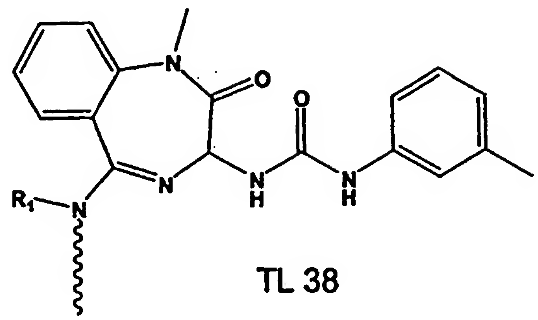
Gastrin/Cholecystokinin Type B Receptor (CCKBR) are enriched on the membranes of a variety of human malignancies including: medullary thyroid cancer, small cell lung cancer, astrocytomas, stromal ovarian cancers, and occasionally in gastroenteropancreatic tumors, breast, endometrial, and ovarian adenocarcinomas. CCKBR are present normally in the stomach, and brain. CCKBR selective ligands coupled to cytotoxin and radionuclides have been described as potential tumor therapeutic and diagnostic agents. However, the potential is severely limited by the expression of CCKBR by normal important tissues The following references relate to this subject matter: Czerwinski G., et al., "Cytotoxic Agents Directed to Peptide Hormone Receptors: Defining the Requirements for a Successful Drug," *Proc Natl Acad Sci USA,* 95(20):11520-5 (1998); de Jong M., et al., "Preclinical and Initial Clinical Evaluation of 111In-Labeled Nonsulfated CCK8 Analog: A Peptide for CCK-B Receptor-Targeted Scintigraphy and Radionuclide Therapy," *J Nucl Med,* 40(12):2081-7 (1999); Behr T.M., et al., "Targeting of Cholecystokinin-B/Gastrin Receptors in Vivo: Preclinical and Initial Clinical Evaluation of the Diagnostic and

Therapeutic Potential of Radiolabelled Gastrin," *Eur J Nucl Med,* 25(4):424-30 (1998); Sinha J., et al., "Quantitative Structure-Activity Relationship Study on Some Nonpeptidal Cholecystokinin Antagonists," *Bioorg Med Chem,* 7(6):1127-30 (1999); Behr T.M., et al., "Radiolabeled Peptides for Targeting Cholecystokinin-B/Gastrin Receptor-Expressing Tumors," *J Nucl Med,* 40(6):1029-44 (1999); Biagini P., et al., "The human Gastrin/Cholecystokinin Receptors: Type B and Type C Expression in Colonic Tumors and Cell Lines," *Life Sci,* 61(10):1009-18 (1997); Reubi J.C.; Waser B., "Unexpected High Incidence of Cholecystokinin-B/Gastrin Receptors in Human Medullary Thyroid Carcinomas," *Int J Cancer,* 67(5):644-7 (1996); Reubi J.C., et al., "Unsulfated DTPA- and DOTA-CCK Analogs as Specific High-Affinity Ligands for CCK-B Receptor-Expressing Human and Rat Tissues in Vitro and in Vivo," *Eur J Nucl Med,* 25(5):481-90 (1998); the contents of which are incorporated herein by reference in their entirety.

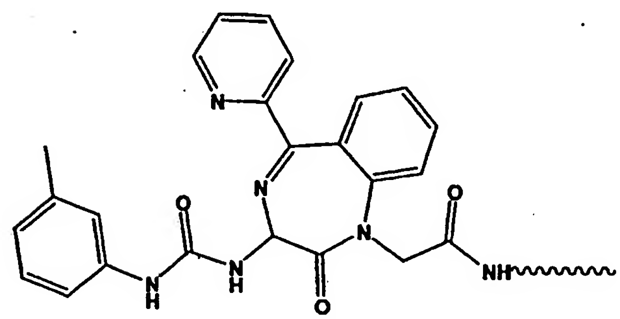
A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to CCKBR.

A large number of groups, which bind to CCKBR with high affinity, are known. A preferred embodiment shown below is based upon the high affinity binding of certain benzodiazepam analog for the CCKBR. The following references relate to this subject matter: Showell G.A., et al., "High-Affinity and Potent, Water-Soluble 5-Amino-1,4-Benzodiazepine CCKB/Gastrin Receptor Antagonists Containing a Cationic Solubilizing Group," *J Med Chem,* 37(6):719-21 (1994), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (TL38 , TL39, andTL40) the targeting ligands comprise the following structures:



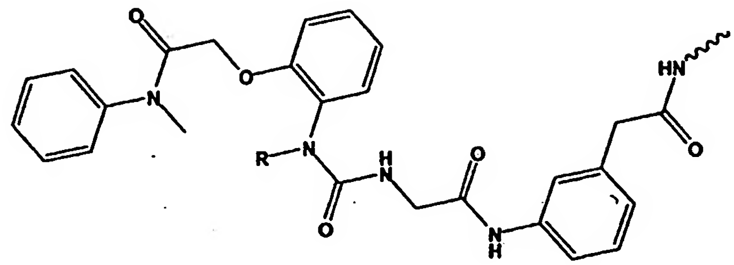
TL 38



TL39

wherein the wavy line is the site of linker attachment, and R_1 is H, or methyl, or ethyl.

The following reference relates to this subject matter: Matassa V.G., "5-(Piperidin-2-yl)- and 5-(Homopiperidin-2-yl)-1,4-benzodiazepines: High-Affinity, Basic Ligands for the Cholecystokinin-B Receptor," *J Med Chem*, 40(16):2491-2501 (1997), the contents of which is incorporated herein by reference in its entirety.



TL40

wherein the wavy line is the site of linker attachment and R is benzyl, cyclohexylmethyl, cyclohexylethyl, cyclohexylpropyl, cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cycloheptylmethyl, 2-methylpropyl, 2,2,dimethylpropyl, 3-methylbutyl, n-butyl, 2-ethylbutyl, 3-methylpentyl, 4-methyl-3-pentenyl, or 4-methylpentyl.

The following reference relates to this subject matter: Takeda Y., et al., "Synthesis of Phenoxyacetic Acid Derivatives as Highly Potent Antagonists of Gastrin/Cholecystokinin-B Receptors. III," *Chem Pharm Bull (Tokyo),* 47(6):755-71 (1999), the contents of which is incorporated herein by reference in its entirety.

Guanidinobenzoatase SelectiveTargeting Ligands

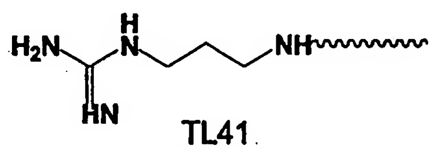
Guanidinobenzoatase GB is protease that is enriched on the surface of most human malignancies. There is evidence that GB is a precursor to a tumor-associated collagenase. Selective stains for GB have been employed to identify malignant cells in pathology specimens. GB binds to a variety of guanidino and

amino analogs. GB selective ligands coupled to mitomycin C and adriamycin have been described as potential anti-cancer agents. However, GB is not specific to malignant cells and as a solo targeting factor is unlikely to provide sufficient tumor selectivity. GB is also present on the surface of normal colonic epithelial cells. The following references relate to this subject matter: Steven F., et al., "A Fluorescent Study of Ligands for Guanidinobenzoatase, a Protease Associated with Tumour Cells," *Anti-cancer Res,* 8(6):1179-83 (1988); Steven F.S., et al., "A Simple Fluorescent Technique for the Location of Tumour Cells in Frozen Sections of the Head and Neck Region," *Anti-cancer Res,* 11(3):1189-94 (1991); Poustis-Delpont C., et al., "Characterization and Purification of a Guanidinobenzoatase: A Possible Marker of Human Renal Carcinoma," *Cancer Res,* 52(13):3622-8 (1992); Anees M.; Benbow E.W. et al., "Dansyl Fluoride, a Fluorescent Inhibitor for the Location of Tumour Cells in Human Tissues," *J Enzyme Inhib,* 10(3):195-201 (1996); Thaon S., et al., "Differential SP220K Expression in Renal Carcinoma and Oncocytoma Cells," *Int J Cancer,* 72(5):752-7 (1997); Steven F.S., et al., "Fluorescent Location of Malignant Cells in Smears Obtained From Sputum," *Anti-cancer Res,* 14(5A):2021-4 (1994); Steven F.S., et al., "Fluorescent Location of Abnormal Cells in Cell Smears Obtained from the Lungs of Patients with Lung Cancer," *Anti-cancer Res,* 12(3):625-9 (1992); Steven F.S., et al., "Fluorescent Location of Tumour Cells in Fine Needle Aspirates," *Anti-cancer Res,* 11(5):1697-9 (1991); Bernstein L.J., et al., "Guanidinobenzoatase and UPA in High-Grade Human Astrocytomas and after Xenografting Cell Suspensions into the Rat Cerebral Cortex: Proteases for Metastasis and Disease Progression," *Anti-cancer Res,* 18(4A):2583-90 (1998); Steven F.S., et al., "GB (Guanidinobenzoatase) Cell Surface Protease and

Serum Inhibitors in Colorectal Neoplasia," *J Pathol*, 167(1):19-24 (1992); Anees M.; Steven F.S., et al., "Inhibition of a Tumour Protease with 3,4-Dichloroisocoumarin, Pentamidine-Isethionate and Guanidino Derivatives," *J Enzyme Inhib*, 8(3):213-21 (1994); Anees M., "Interaction of Tissue Plasminogen Activator Inhibitor with Cell Surface Guanidinobenzoatase and Urokinase Plasminogen Activator," *J Enzyme Inhib*, 10(4):281-8 (1996); Steven F.S., et al., "Labelling of Tumour Cells with a Biotinylated Inhibitor of a Cell Surface Protease," *J Enzyme Inhib*, 4(4):337-46 (1991); Anees M., "Location of Tumour Cells in Colon Tissue by Texas Red Labelled Pentosan Polysulphate, an Inhibitor of a Cell Surface Protease," *J Enzyme Inhib*, 10(3):203-14 (1996); Steven F.S., et al., "Studies on the Activity of a Protease Associated with Cells at the Advancing Edge of Human Tumour Masses in Frozen Sections," *Br J Cancer*, 58(1):57-60 (1988); Steven F.S., et al., "Targeting Adriamycin to Tumour Cells by Means of an Affinity Ligand; A Model System for Drug Delivery," *Anti-cancer Res*, 9(1):247-53 (1989); Steven F.S., et al., "The Targeting of Agmatine-Liganded Mitomycin C to an Enzyme on the Surface of Tumour Cells," *Anti-cancer Res*, 10(3):583-9 (1990); Steven F.S., et al., "The Design of Fluorescent Probes which Bind to the Active Centre of Guanidinobenzoatase. Application to the Location of Cells Possessing this Enzyme," *Eur J Biochem*, 149(1):35-40 (1985); Poustis-Delpont C., et al., "Monomeric 55-kDa Guanidinobenzoatase Switches to a Serine Proteinase Activity upon Tetramerization. Tetrameric Proteinase SP 220 K Appears as the Native Form," *J Biol Chem*, 269(20):14666-71 (1994), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to GB.

In preferred embodiment (TL41) the targeting ligand comprises the following structure:



TL41

wherein the wavy line is the site of linker attachment.

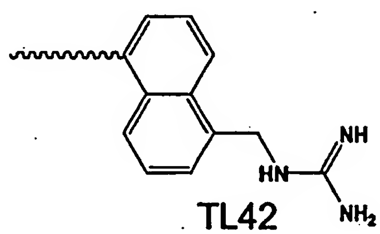
Norepinephrine Transporter Selective Ligands

A variety of neuroendocrine malignancies including neuroblastoma, and malignant pheochromocytomas have increased expression of the norepinephrine transporter (NET). M-Iodobenzylguanidine, which binds to NET, has been utilized for diagnosis and therapy of NET+ malignancies. Fluorescent analogs have also been described as potential diagnostic aids. The following references relate to this subject matter: Nakagami Y., et al., "A Case of Malignant Pheochromocytoma Treated with 131I-Metaiodobenzylguanidine and Alpha-Methyl-P-Tyrosine," *Jpn J Med*, 29(3):329-33 (1990); Smets L.A., et al., "Extragranular Storage of the Neuron Blocking Agent Meta-Iodobenzylguanidine (MIBG) in Human Neuroblastoma Cells," *Biochem Pharmacol*, 39(12):1959-64 (1990); Gelfand M.J., et al., "Meta-Iodobenzylguanidine in Children," *Semin Nucl Med*, 23(3):231-42 (1993); Hadrich D., et al., "Synthesis and Characterization of Fluorescent Ligands for the Norepinephrine Transporter: Potential

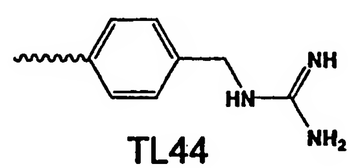
Neuroblastoma Imaging Agents," *J Med Chem*, 42(16):3101-8 (1999); Beierwaltes W.H., et al., "Update on Basic Research and Clinical Experience with Metaiodobenzylguanidine," *Med Pediatr Oncol*, 15(4):163-9 (1987), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention is a compound ET with a targeting ligand comprised of a structure that binds to the norepinephrine transporter.

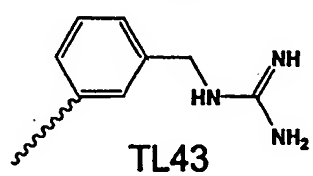
In preferred embodiments (TL42 , TL43, and TL44) the targeting ligands comprise the following structures:



TL42



TL44



TL43

wherein the wavy line is the site of linker attachment.

Monoclonal Antibody Based Multifunctional Drug Delivery Vehicles

Monoclonal antibody–toxin conjugates are well known. It has been 25 years since the landmark development of monoclonal antibodies by Kohler and Milstein. The following reference relates to this subject matter: Köhler G.; Milstein C., "Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity," *Nature,* 256:495-497 (1975), the contents of which is incorporated herein by reference in its entirety.

Despite enormous efforts, only a handful of monoclonal antibody based drugs have been approved for clinical use. Monoclonal antibodies offer the promise of exquisite targeting specificity. However, there are actually very few antigenic targets known which are *absolutely* specific for malignant cells. Attempts to utilize anti-Lewis Y monoclonal antibodies conjugated to the anti-cancer drug doxorubicin illustrate the problem. The Lewis Y antigen is an excellent tumor-associated antigen, which is enriched on the majority of epitheal malignancies including: breast cancer, colon cancer, non small cell lung cancer, cervical cancer, ovarian cancer and melanoma. The conjugate known as Br96-Doxorubicin, when evaluated in women with metastatic breast cancer, was found to be less effective than non-targeted doxorubicin and to have gastrointestinal toxicity. The Lewis Y antigen is present in parts of the GI tract and resulted localization of the toxin to the gastric mucosa that is believed to have the GI toxicity. The following references relate to this subject matter: Saleh M.N., et al., "Phase I Trial of the Anti–Lewis Y Drug Immunoconjugate BR96-Doxorubicin in Patients with Lewis Y–Expressing Epithelial Tumors," *J Clinical Oncology,* 18(11):2282-2292 (2000); Tolcher,A.W. et al., "Randomized Phase II

Study of BR96-Doxorubicin Conjugate in Patients With Metastatic Breast Cancer" *J Clinical Oncology,* 17(2):478 (1999), the contents of which are incorporated herein by reference in their entirety.

The fundamental problem is that a single factor (in this case the Lewis Y antigen) is not sufficient to distinguish malignant cells from normal cells. Anti-cancer drugs need to be multifactorial. The present invention can enable multifactorial targeting. For example, a drug targeted against cells that only express both the Lewis Y antigen and urokinase would provide exquisite selectivity for breast cancer cells. Urokinase is not present in the GI tract.

Targeting specificity alone is often not sufficient to achieve therapeutic effect. The present invention can be employed to enhance the function of monoclonal antibodies including the ability to:

1.) Enhance the affinity of binding to the target cells;

2.) Enhance the selectivity of target cell binding;

3.) Enhance uptake by target cells;

4.) Allow detoxification by non-target cells;

5.) Overcome multi-drug resistance; and

6.) Ameliorate the problem of antibody inactivation by the binding of soluble antigens.

The present invention encompasses a compound ET; wherein T is a targeting agent that binds or interacts with the target cell or its microenvironment and E is one or more effector moieties that effect the desired chemical, physical, or

biological activity; and wherein T is comprised of two or more groups such that each functionality independently and specifically enhances targeting selectivity, affinity, specificity, drug activation, intracellular transport, intracellular localization, or drug detoxification; and wherein one of the groups that comprise T is a monoclonal antibody or targeting receptor binding fragment of a monoclonal antibody, or an analog or derivative thereof which bears amino acid sequence similarity to ligand binding portion of a monoclonal antibody or a fab fragment of an antibody.

A preferred embodiment is comprised of the compound ET in which E is comprised of one or more effector agents having pharmacological activity designated as "PA" and wherein T comprises:

a) A group referred to as a "targeting ligand" which selectively binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell; and wherein this targeting ligand is comprised of: a monoclonal antibody or targeting receptor binding fragment of a monoclonal antibody, or an analog or derivative thereof which bears amino acid sequence similarity to portions of a monoclonal antibody; or a natural protein, or a complex of natural proteins, or a protein, or a naturally occurring polymer; and

b) One or more of the following:

I. A second targeting ligand which selectively binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell;

II. A group, referred to as a "masked intracellular transport ligand" which can be modified in vivo to give a group referred to as an "intracellular transport ligand" which binds to a target cell receptor that actively transports bound ligands into the target cell;

III. A group referred to as a "trigger" that can be modified in vivo, wherein in vivo modification activates the trigger and modulates the pharmacological activity PA;

IV. or a group referred to as a "masked intracellular trapping ligand" which can be modified in vivo to give an "intracellular trapping ligand"; that can bind to one or more type of intracellular receptor;

and wherein if a second targeting ligand is present in T then the first and second targeting ligands are able to bind simultaneously to two targeting receptor molecules;

and wherein if T consists solely of a targeting ligand a trigger and in vivo modification of the trigger increases the pharmacological activity PA then the in vivo modification which activates the trigger is caused by an enzyme or enzymatic activity that is increased at target cells or decreased at non-target cells;

and wherein if T consists solely of a targeting ligand a trigger and in vivo modification of the trigger decreases the pharmacological activity PA then the in vivo modification which activates the trigger is caused by an enzyme or enzymatic activity that is decreased at target cells or increased at non-target cells;

and provided that T is not: an antibody, or an analog or component of an antibody, or a complex of antibodies, or a bispecific antibody, or an analog of a bispecific antibody, or a natural protein, or a complex of natural proteins, or a protein, or a naturally occurring polymer.

Affinity of the targeting complex to the cell can be increased by having a targeting ligand that also binds to a cell associated receptor. Target selectivity can be enhanced and modified by the increased rate and affinity of binding that can occur with the addition of a second binding ligand designated "A2". This can be especially useful if the density of antigen on the target cells is low while the density of receptors to A2 is high. Binding of a monoclonal antibody to a cell does not insure that effective internalization can take place. Several embodiments of the present invention can be used to insure effective intracellular delivery. One approach is to employ a second targeting ligand A2 that binds to receptors on tumor cells and undergoes receptor mediated endocytosis. The use of a masked intracellular transporter ligand, as discussed previously, can allow for efficient cell uptake without compromising the targeting selectivity. The incorporation of a detoxification trigger allows for the option to selectively inactivate the drug in non-target cells. For example, a monoclonal antibody enzyme conjugate can be targeted to antigens present on critical non-target cells and can selectively detoxify drug at this site. As discussed previously, multi-drug resistance can be overcome by incorporating, in the effector portion of the drug, an inhibitor to P-glycoprotein. The simultaneous coupling of different antineoplastic drugs can also decrease the emergence of drug resistance. The present invention can also be used to ameliorate the

problem of soluble antigen interfering with cell binding. The rate at which antigen-antibody binds, is a function of the concentration of the antigen. A second targeting ligand A2 with high affinity for a target cell receptor can increase the concentration of the antibody at the surface of the target cell and consequently increase the rate at which the antibody binds to target cell associated antigen. In addition, even if the antibody has complexed antigen in circulation, A2 can still localize the drug to target cells. Exchange of the soluble antigen with cell bound antigen can be favored by the higher concentration of the antigen on the cell membrane.

The scope of the present invention includes a method to increase the selectivity and binding affinity of monoclonal antibodies, antibody analogs (and other proteins or factors) for targets by coupling to the monoclonal antibody one or more targeting ligands that bind to independent receptors on the intended target and also the method of coupling to the monoclonal antibody one or more groups of the structure E-T. The scope of the present invention also includes the compounds that result from the coupling of ET and an antibody or other protein or natural product that can benefit from the enhanced targeting selectivity, binding affinity, intracellular transport or trapping possible with multifunctional drug delivery vehicles ET of the present invention.

The scope of the present invention also includes a method to increase the intracellular delivery of monoclonal antibodies, antibody analogs (and other proteins or factors) by coupling to the monoclonal antibody a masked intracellular transporter ligand.

Affinity of the targeting complex to the cell can be increased by having a group A2 that also binds to a cell associated receptor. Target selectivity can be enhanced and modified by the increased rate and affinity of binding that can occur with the addition of a second binding ligand A2. This can prove especially useful if the density of antigen on the target cells is low while the density of receptors to A2 is high. Binding of a monoclonal antibody to a cell does not insure that effective internalization can take place. Several embodiments of the present invention can be used to insure effective intracellular delivery. One approach is to employ a second binding ligand A2 that is known to bind to receptors on tumor cells and undergoes receptor mediated endocytosis. The use of a masked intracellular transporter ligand, as discussed previously, can allow for efficient cell uptake without compromising the targeting selectivity. The incorporation of a detoxification trigger allows for the option to selectively inactivate the drug in non-target cells. For example, a monoclonal antibody enzyme conjugate can be targeted to antigens present on critical non-target cells and can selectively detoxify drug at this site. As discussed previously, multi-drug resistance can be overcome by incorporating, in the effector portion of the drug, an inhibitor to P-glycoprotein. The simultaneous coupling of different antineoplastic drugs can also decrease the emergence of drug resistance. The present invention can also be used to ameliorate the problem of soluble antigen interfering with cell binding. The rate at which antigen-antibody binds is a function of the concentration of the antigen. A second receptor A2 with high affinity for a target cell receptor can increase the concentration of the antibody at the surface of the target cell and consequently increase the rate at which the antibody binds to target cell associated antigen. In addition, even if the antibody

has complex antigen in circulation, A2 can still localize the drug to target cells. Exchange of the soluble antigen with cell bound antigen can be favored by the higher concentration of the antigen on the cell membrane.

The scope of the present invention includes a method to increase the selectivity and binding affinity of monoclonal antibodies, antibody analogs (and other proteins or factors) for targets by coupling to the monoclonal antibody one or more targeting ligands that bind to independent receptors on the intended target and also the method of coupling to the monoclonal antibody one or more groups of the structure E-T.

The scope of the present invention also includes a method to increase the intracellular delivery of monoclonal antibodies, antibody analogs (and other proteins or factors) by coupling to the monoclonal antibody a masked intracellular transporter ligand.

Linkers

A large variety of chemical structures can be employed as linkers. Considerations for the selection of linkers designated as "L" are as follows:

1) L should have chemical groups that allow it to be covalently coupled to the components of the compound ET. The covalently coupling preferably should not significantly interfere with the function of the attached components;

2) For some but not all embodiments, L should be of sufficient length to allow for crosslinking of targeting receptors;

3) L can preferably be inert in the sense that L should generally not bind with high affinity to cells or tissue components;

4) L should be sufficiently chemically stable to allow the drug to reach its target site functionally intact;

5) L can also have sites to which groups that allow manipulation of drug solubility can be attached; and

6) L preferably should have low immunogenicity.

Linkers with water solubility are especially preferred. Similar requirements apply to linkers used to couple other components of the drug molecule together. The optimal length of the linkers can vary depending on the structure of the receptors. The expected range is from one up to about 350 bond lengths or from 1 to about 10 bond lengths, or from about 10 to about 40 bond lengths, or from about 20 to about 80 bond lengths, or from about 80 to about 150 bond lengths, or from about 150 to about 350 bond lengths, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14....350 or about 350 bond lengths; wherein the dots are used to represent the individual numbers in the sequence between 14 and 350. The linkers can be comprised of oligo or poly-ethylene glycols –(O-CH2-CH2-)n- with (n=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11...or 120 or about 120), gycols, oligo or polypropylene glycols, polypeptides, oligopeptides, -(CH2)n-, with (n=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11...or 25 or about 25). The linker can have groups that increase water solubility. Preferred embodiments of such groups comprise: phosphates, phosphonates, phosphinates, sulfonates, carboxylates, amines, hydroxy groups, and polyalcohols. The linker can be connected to the other components of ET by a large variety of chemical bonds. Preferred functionalities

include, but are not limited to: carboxylate esters and amides, amides, ethers, carbon- carbon, disulfides, -S-S-S-, acetals, esters of phosphates, esters of phosphinates, esters of phosphonates, carbamates, ureas, N-C bonds, thioethers, sulfonamides, and thioureas. Especially preferred are amide bonds.

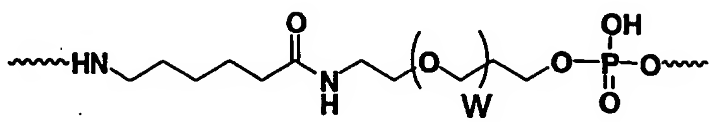
Linkers can be linear or can be nonlinear with branches. Linkers can be comprised of shorter linkers that are covalently joined. In preferred embodiments the covalent joining is at a multivalent molecule to which multiple linkers can be coupled. The multivalent molecule can be essentially any molecule to which the linkers can be covalently coupled. Preferred embodiments are molecules that have multiple chemical functionalities such as amino, carboxylate, hydroxy, -SH, isocyanate, and isothiocyanate that can be reacted with the linker to form a covalent bond. Preferred embodiments include: L-amino acids, D- amino acids, or racemic mixtures thereof, amino acid analogs, lysine, aspartic acid, cysteine, glutamic acid, serine, homoserine, hydroxyproline, ornithine, tyrosine, glycerol, pentaerithrol, erithol, and citric acid. One skilled in the arts would readily recognize a very large number of other polyfunctional molecules that can be employed to connect smaller linkers together.

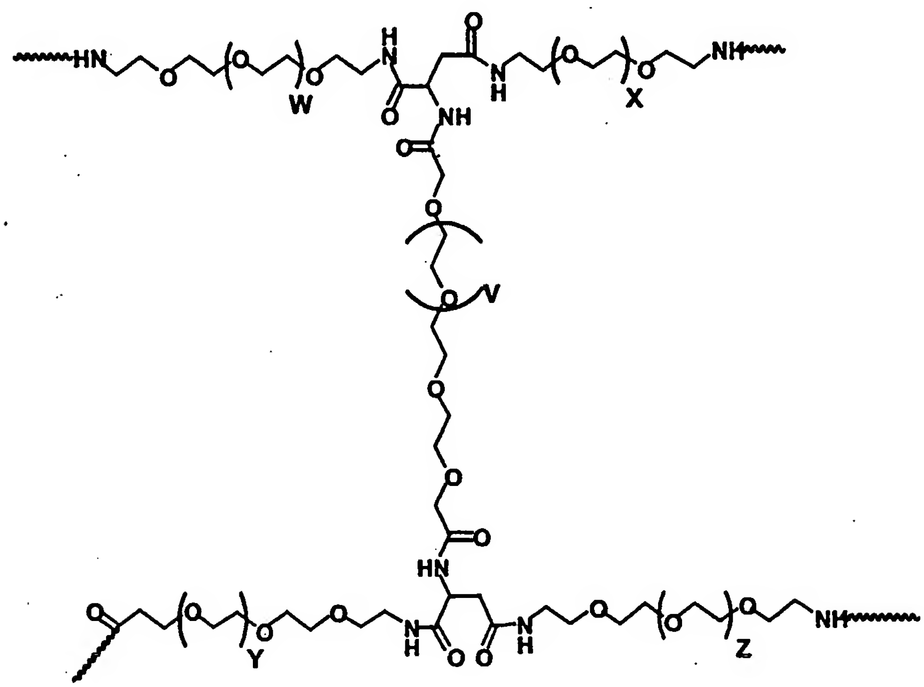
Examples of molecules that are suitable for use as linkers or as molecules to join together multiple linkers can be found in the Aldrich Chemical Catalog (2000) of Sigma –Aldrich Co. and the Shearwater Polymers, Inc. Catalog "Functionalized Biocompatible Polymers for Research and Pharmaceuticals. Polyethylene Glycol and Derivatives," (2000), and Calas M., et al., "Antimalarial Activity of Compounds Interfering with *Plasmodium falciparum* Phospholipid

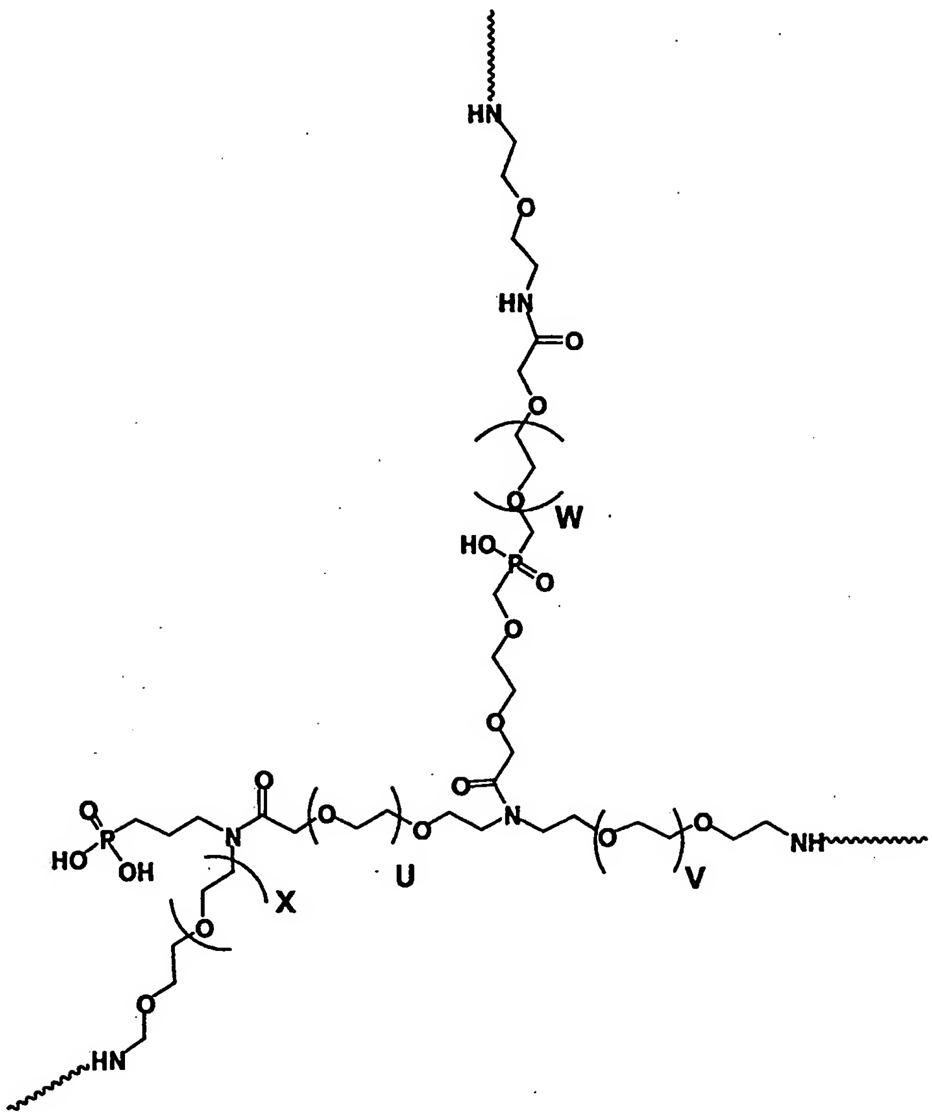
Metabolism: Comparison between Mono- and Bisquaternary Ammonium Salts," *J Med Chem,* 43:505-516 (2000); and Girault S., et al., "Antimalarial, Antitrypanosomal, and Antileishmanial Activities and Cytotoxicity of Bis(9-amino-6-chloro-2-methoxyacridines): Influence of the Linker," *J Med Chem,* 43:2646-2654 (2000); and Halazy S., et al., "Serotonin Dimers: application of the Bivalent Ligand Approach to the Design of New Potent and Selective 5-HTAgonists," *J Med Chem,* 39:4920-4927 (1996); and Yano K., et al., "Simultaneous Activation of Two Different Receptor Systems by Enkephalin/Neurotensin Conjugates having Spacer Chains of Various Lengths," *Eur J Pharm Sci,* 7:41-48 (1998);and Profit A.A., et al., "Bivalent Inhibitors of Protein Tyrosine Kinases," *J Am Chem Soc,* 121:280-283 (1999)and Portoghese P.S., et al., "Hybrid Bivalent Ligands with Opiate and Enkephalin Pharmacophores," *J Med Chem,* 30:1991-1994 (1987); and Glick G.D., et al., "Ligand Recognition by Influenza Virus," *J Biol Chem,* 266(35):23660-23669 (1991); Glick J.D.; Knowles J.R., "Molecular Recognition of Bivalent Sialosides by Influenza Virus," *J Am Chem Soc,* 113:4701-4703 (1991);and Blaustein R.O., et al., "Tethered Blockers as Molecular 'Tape Measures' for a Voltage-Gated K+ Channel," *Nature Structural Biology,* 7(4):309-311 (2000); Tetsui S., et al., "Opioid Receptor Affinity of Multivalent Ligand System Consisting of Polymerized Liposome," *Int J Peptide Protein Res,* 48:95-101 (1996); and Sasaki-Yagi Y., et al., "Binding of Enkephalin/Dextran Conjugates to Opioid Receptors," *Int J Peptide Protein Res,* 43:219-224 (1994); and ; and Zhao J., et al., "Receptor Affinity of Neurotensin Message Segment Immobilized on Liposome," *Biochimica et Biophysica Acta,* 1282:249-256 (1996) and Kane P., et al., "Cross-Linking of IgE-Receptor Complexes at the Cell Surface: Synthesis and Characterization of a Long

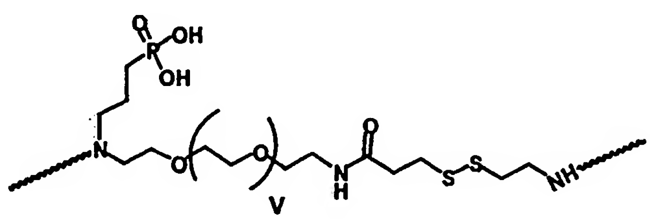
Bivalent Hapten that is Capable of Triggering Mast Cells and Rat Basophilic Leukemia Cells," *Molecular Immunology,"* 23(7):783-790 (1986); and WO 99/53951 10/28/99 Martinez, et al., "Terminally-Branched Polymeric Linkers and Polymeric Conjugates Containing the Same".; and 5,783,1787/21/98 and Rose K.; Vizzavona J., "Stepwise Solid-Phase Synthesis of Polyamides as Linkers," *J Am Chem Soc,* 121:7034-7038 (1999); and Kramer R.H.; Karpen J.W., "Spanning Binding Sites on Allosteric Proteins with Polymer-Linked Ligand Dimers," *Nature,* 395:710-713 (1998); and Fan E., et al., "High-Affinity Pentavalent Ligands of *Escherichia coli* Heat-Labile Enterotoxin by Modular Structure-Based Design," *J Am Chem Soc,* 122:2663-2664 (2000); and Riley A M.; Potter B.V.L., "Poly(ethylene glycol)-linked Dimers of D-*myo*-inositol 1,4,5-trisphosphate," *Chem Commun,* 983-984 (2000); and Rajur S.B., et al., "Hoechst 33258 Tethered by a Hexa(ethylene glycol) Linker to the 5'-Termini of Oligodeoxynucleotide 15-Mers: Duplex Stabilization and Fluorescence Properties," *J Org Chem,* 62:523-529 (1997); and Schwabacher A.W., et al., "Desymmetrization Reactions: Efficient Preparation of Unsymmetrically Substituted Linker Molecules," *J Org Chem,* 63:1727-1729 (1998); and Bertozzi C. R.; Bednarski M.D., "The Synthesis of Heterobifunctional Linkers for the Conjugation of Ligands to Molecular Probes," *J Org Chem,* 56:4326-4329 (1991); and Sépulchre M., et al., "Specific Functionalization of Polyoxirane by Amino, Carboxyl, Sulfo, and Halogeno End Groups," *Makromol Chem,* 184:1849-1859 (1983); and Cook R.M., et al., "The Preparation and Synthetic Application of Heterobifunctional Biocompatible Spacer Arms," *Tetrahedron Letters,* 35(37):6777-6780 (1994), the contents of which are hereby incorporated by reference in their entirety.

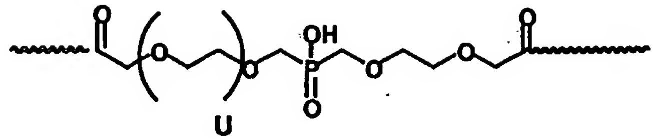
Some preferred embodiments of linkers are shown below:

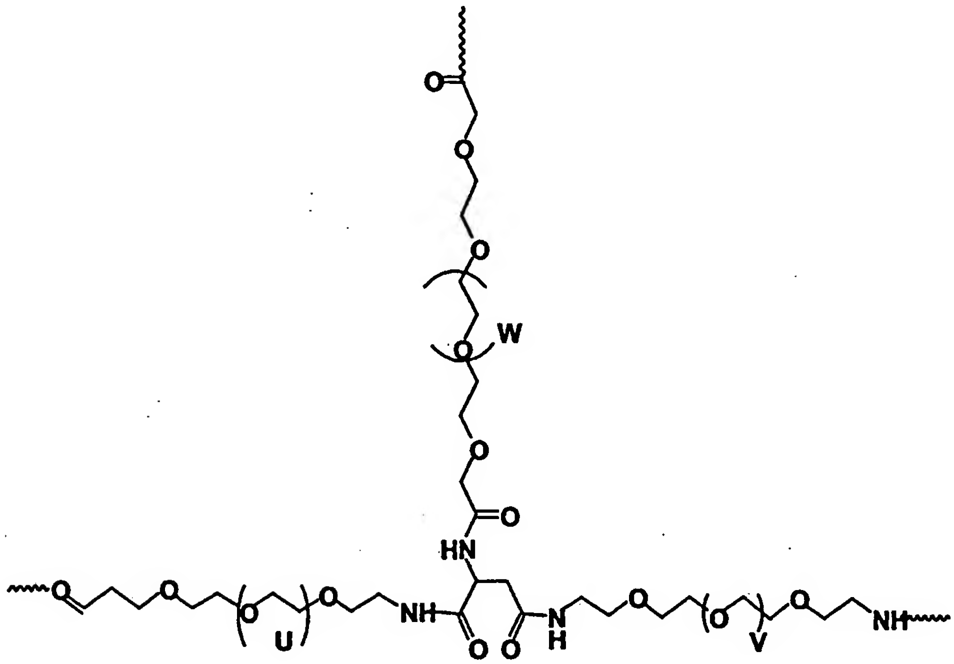


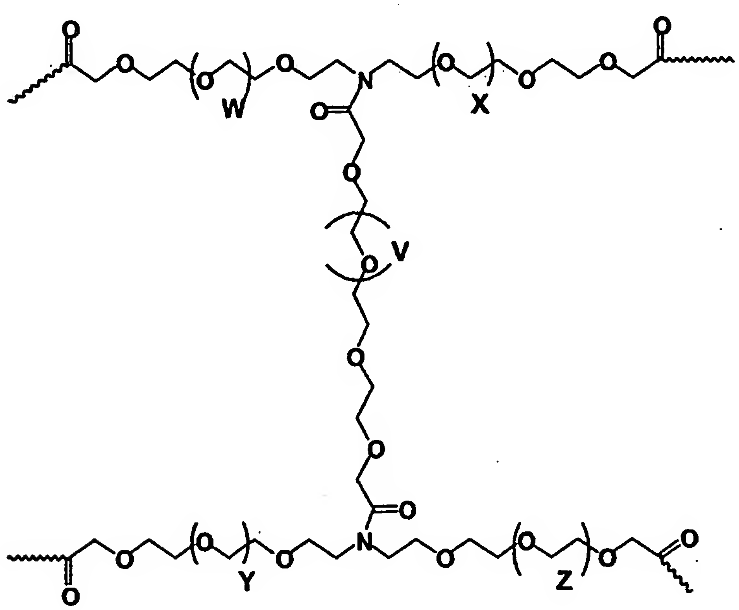


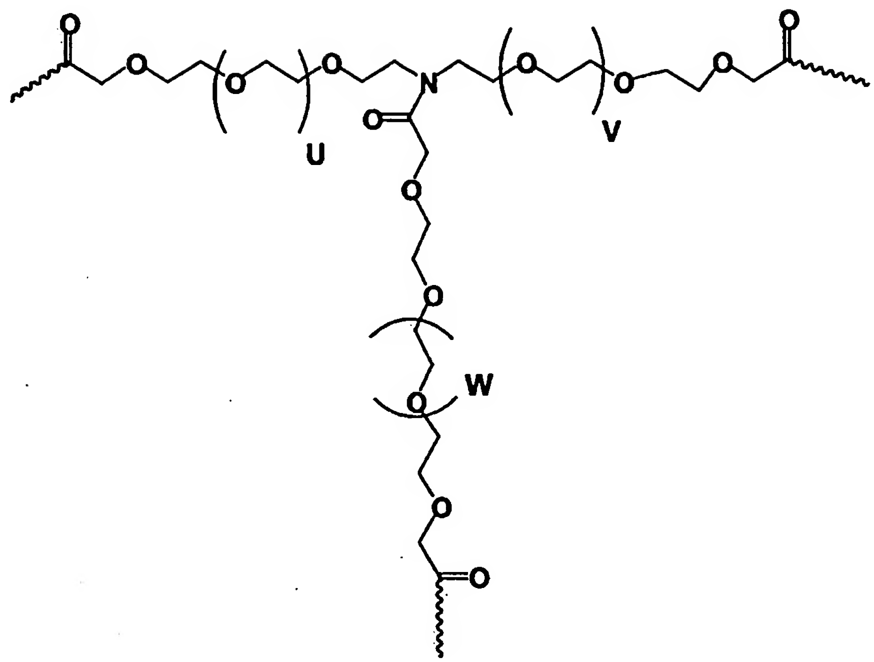


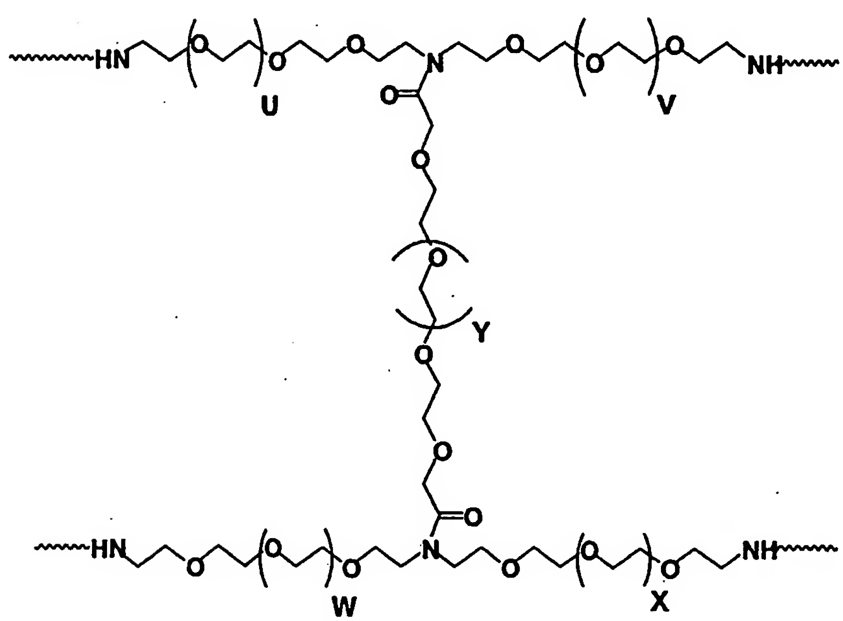


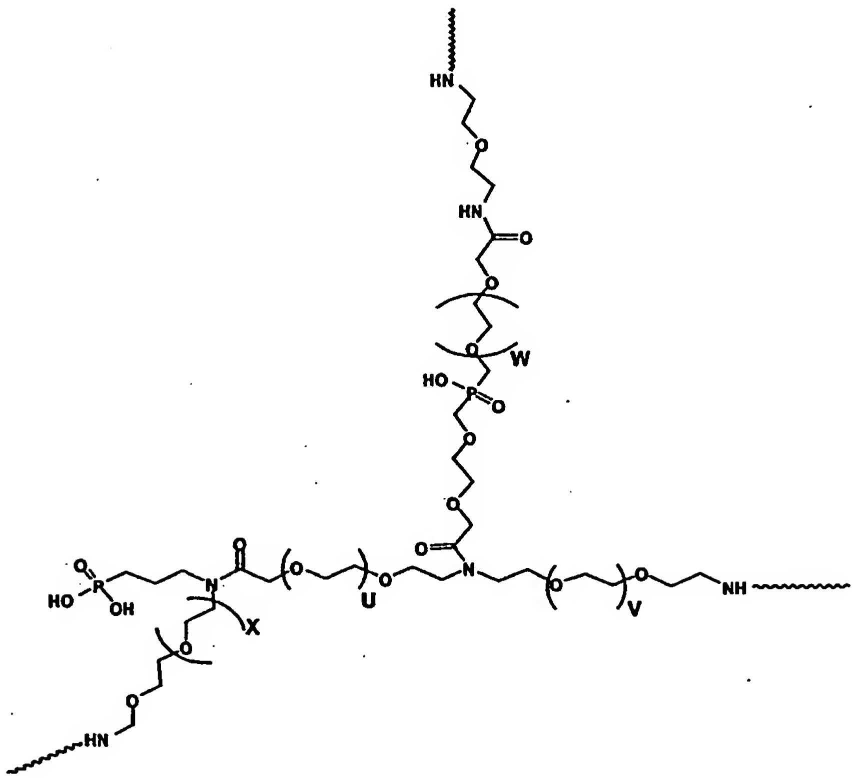


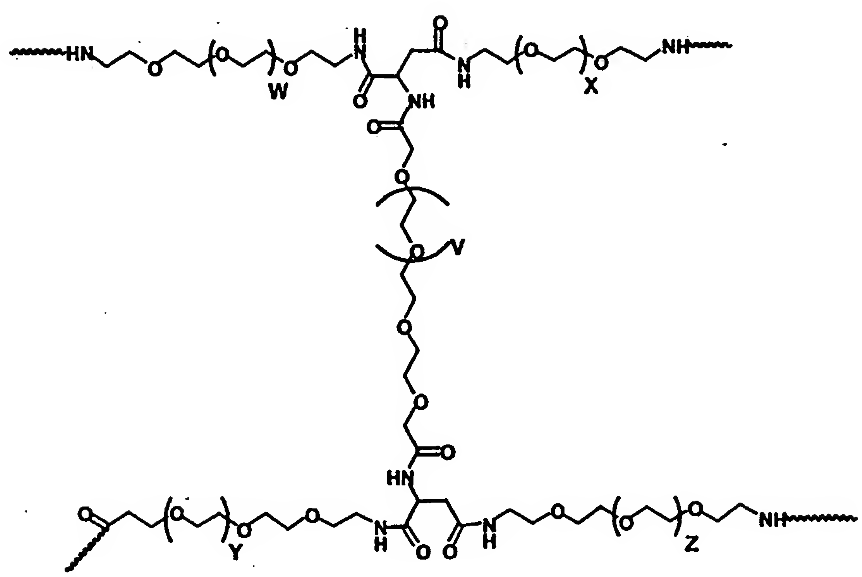





O
O
O
O
O
N
O
O
O
U
V
O
O
O
O
W
O
O
O


HN
O
O
O
N
O
O
O
NH
U
V
O
O
O
Y
O
O
O
HN
O
O
O
N
O
O
O
NH
W
X





where U= 0, 1, 2, 3, 4, 5, 6, ...150 or about 150;

where V= 0, 1, 2, 3, 4, 5, 6, ...150 or about 150;

where w= 0, 1, 2, 3, 4, 5, 6, ...150 or about 150;

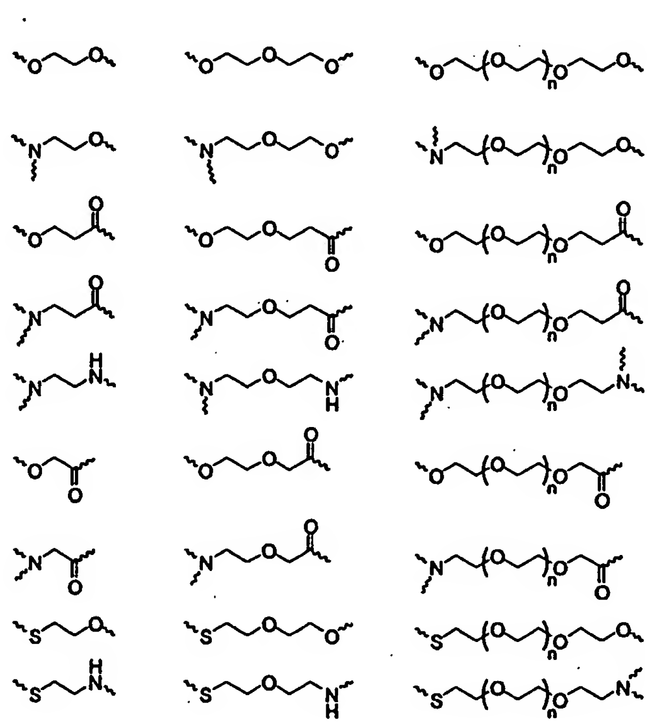
where x= 0, 1, 2, 3, 4, 5, 6, ...150 or about 150;

where y= 0, 1, 2, 3, 4, 5, 6, ...150 or about 150;

where z= 0, 1, 2, 3, 4, 5, 6, ...150 or about 150;

and wherein the wavy lines are the sites of attachment of the linkers to other components of ET.

Additional preferred embodiments of linkers are comprised of the following structures:



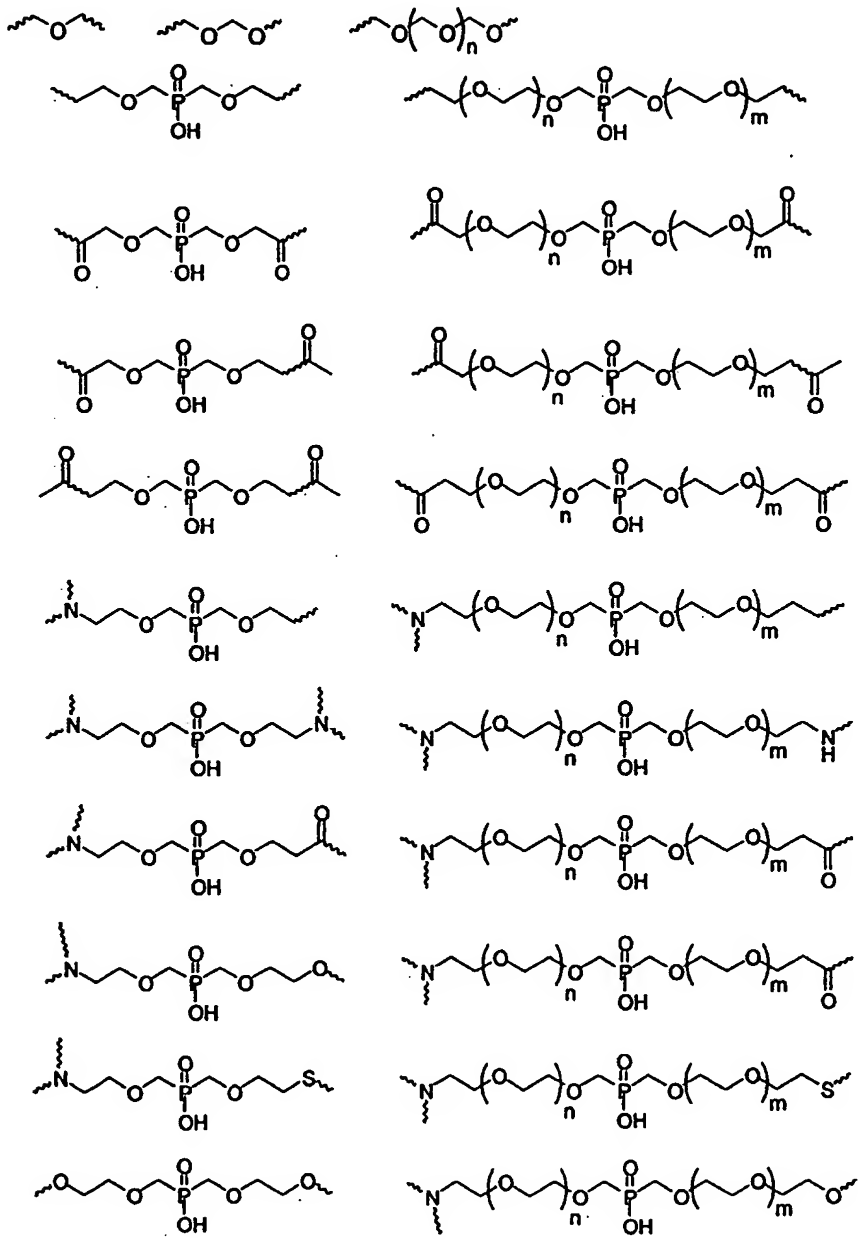
~CH_2~ ~CH_2-CH_2~ ~CH_2-CH_2-CH_2~ ~$CH_2(CH_2)_nCH_2$~

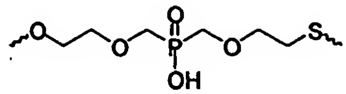
~CH_2-CO~ ~CH_2-CH_2-CO~ ~CH_2-CH_2-CH_2-CO~ ~$CH_2(CH_2)_nCH_2$-CO~

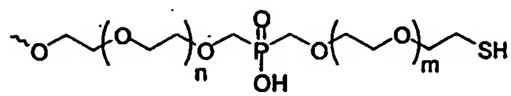
~N—CO~ ~N—CH_2-CO~ ~N—CH_2-CH_2-CO~ ~N—$(CH_2)_nCH_2$-CO~

~O—CO~ ~O—CH_2-CO~ ~O—CH_2-CH_2-CO~ ~O$(CH_2)_nCH_2$-CO~

—O— ~CH_2O~ ~CH_2-CH_2O~ ~$CH_2(CH_2)_nCH_2$O~







wherein the wavy line is the site of linker attachment to the components of ET or may be H, and wherein m = 0, 1 , 2, 3, 4, 5, 6, ...150 or about 150; and wherein n = 0, 1 , 2, 3, 4, 5, 6, ...150 or about 150; and wherein the linkers can also be connected to each other or to multifunctional joiner molecules as described above.

Triggers

A wide variety of triggers can be employed in the drug. The function of the triggers is to cause a change in drug function either directly or indirectly by changing the chemistry of the drug upon in vivo modification.Trigger activation can be spontaneous or enzyme catalyzed. Enzyme activated triggers can be non-selective or selective. Selectivity can be for enzymes enriched on target cells or enriched on non-target cells. The triggers can undergo either extracellular or intracellular activation. The triggers can lead to immediate or delayed alteration in drug functionality depending upon the rate of the reaction that is initiated by the triggering event.

The triggers can be attached to the drug in a variety of manners. The key requirements for triggers are as follows:

1.) The trigger can be attached to E-T in a manner that allows for the intended change in drug function upon activation; and

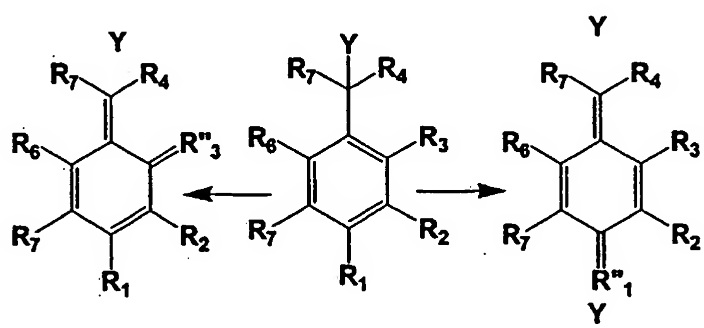
2.) The binding affinity of the trigger, to its activating enzymes, can be much lower than the affinity of the drug to target cells.

In a preferred embodiment, toxifying triggers are designed to undergo cleavage intracellularly and thereby release then free toxins. Intracellular triggers can be activated by a wide range of intracellular enzymes including: hydrolases, proteases, amidases, glycoside hydrolases, thioreductases, Glutathione-S-Transferases, nitroreductases, oxidases, phosphodiesterases, quinone reductases, phosphatases, thiolesterases, oxidoreductases, sulfatases, and esterases.

Note: For the sake of clarity the trigger groups shown in this section include an attached moiety that is released upon trigger activation or trigger function. Strictly speaking, the released group is not part of the trigger group.

In a preferred embodiment (designated TR1) the trigger is comprised of a substituted benzylic analog with a masked or latent electron donating group in the ortho or para positions. Unmasking of this group triggers cleavage of the bond between the benzylic carbon and a leaving group as shown below:

TR1



wherein Y is a leaving group and R_1 and R_3, either alone or both, are groups which can be transformed into electron donating groups designated as $R"_1$ and $R"_3$ by spontaneous or enzymatic chemical processes; and wherein R_1, R_2, R_3, R_4, R_5, R_6, and R_7 can be a wide range of groups including hydrogen, alkyl groups, halogens, alkoxy, -CO-R_8, where R_8 is OH, an alkyl alkoxy group, or where R_8 can be such that COR$_8$ comprises an amide. Groups R_1- R_8 also can have an attachment site for a linker attached to the site on the remainder of the drug E-T. A site for linker attachment is optional and is not needed in all embodiments of the invention. If a linker is needed it can be attached in a manner that does not impede trigger function. At least one of the groups R_1 and R_3 must be capable of transformation or biotransformation into an electron donating group. R_1 and R_3 can be an ester, amide, thioester, disulfide, nitro group, H, azido, phosphoester, phosphonoester, phosphinoester, sulfate, alkoxy group, an amino group that is phosphonylated, or phosphorylated and enol ether, an acetal group, a carbonate, or a carbamate. For a detailed discussion of this type of trigger see: Carl, P., "A Novel Connector Linkage Applicable in Prodrug Design," *J Med Chem,* 24(5):479-480 (1981); 5,627,165, 5/06/97, Glazier, "Phosphorous Prodrugs and Therapeutic Delivery Systems Using Same"; 5,274,162, 12/28/93, Glazier, "Antineoplastic Drugs with Bipolar Toxification/Detoxification Functionalities"; 5,659,061, 8/19/97, Glazier, "Tumor Protease Activated Prodrugs of Phosphoramide Mustard Analogs with Toxification and Detoxification Functionalities"; Senter, Peter D., et al., "Development of a Drug-Release Strategy Based on the Reductive Fragmentation of Benzyl Carbamate Disulfides," *J Org Chem,* 55:2975-2978

(1990), the contents of which are incorporated herein by reference in their entirety.

The table below summarizes some of the groups that are suitable for R_1 and R_3 and the electron donating derivatives into which they are transformed. The mechanisms of the transformation are also shown.

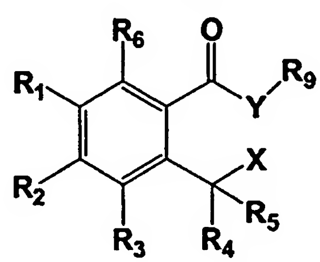
Table 1

Group R1 or R3	Electron Donating Derivative	Mechanism
esters	Hydroxy, oxy anion	esterases
amides	amino	Amidases, proteases
thioesters	Thiol, sulfide anion,	Thioesterases, esterases
disulfides	Thiol, sulfide anion	Thioreductases
nitro	Amino, hydroxyamino	Nitro reductases
azido	amino	Azido reductase
phosphate	Hydroxy, oxy anion	phosphatases
phosphodiesters	Hydroxy, oxy anion	phosphodiesterases
phosphonoesters	Hydroxy, oxy anion	phosphodiesterases
phospinoesters	Hydroxy, oxy anion	hydrolysis
sulfate	Hydroxy, oxy anion	sulfatase
alkoxy	Hydroxy, oxy anion	oxidases
phosphoramides	amino	Hydrolysis
phosphonoamides	amino	hydrolysis
enol ether	Hydroxy, oxy anion	hydrolysis
acetals	Hydroxy, oxy anion	Acid catalyzed, or enzymatic
carbonates	Hydroxy, oxy anion	esterases
carbamates	amino	Oxidative N-dealkylation
hydrogen	Hydroxy, oxy anion	hydroxylation
phosphotriester	Hydroxy, oxy anion	See text

Another preferred embodiment of the trigger utilizes a masked nucleophile which when unmasked catalyzes an intramolecular reaction. The following references relate to this subject matter: Nielsen, N.M. and Bundgaard, H., "Bioreversible Derivatization of Peptides," *Int J Pharm,* 29(9):49-68 (1986); Cain, B.F., "2-Acyloxymethylbenzoic Acids. Novel Amine Protective Functions Providing Amides with the Lability of Esters," *J Org Chem,* 41(11): 2029-2031 (1976); Chiong, K.N.G., et al., "Rationalization of the Rate of the Acylation Step in Chymotrypsin-Catalyzed Hydrolysis of Amides," *J Am Chem Soc,* 97(2):418-423 (1975), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of a trigger (TR2) is a group comprised of the following structure:

TR2

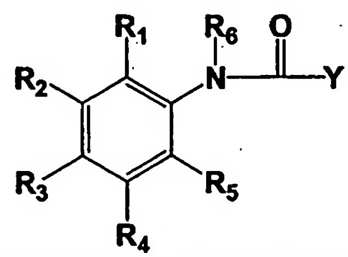


wherein Y is a N bearing group such as NH, NHR7 where R7 is a lower alkyl group which may be substituted with inert groups, or an –S- group, or an –O- group, and HY-R_9 is the compound which is freed upon activation of the trigger, and X is a masked nucleophile and can be a masked amino, hydroxy, or thiol group. R_1-R_6 can be a wide range of groups including: hydrogen, alkyl groups, halogens, Cl, I. F. Br, alkoxy, and -CO-R_8; where R_8 is OH, a lower alkoxy group, or where R_8 can be such that COR_8 comprises an amide. Groups R_1- R_8

also can bear an attachment site for a linker attached to the site on the remainder of the drug. The masked nucleophile X can be any of the groups described in Table 1.

Another preferred embodiment (embodiment TR3) of a trigger is shown below:

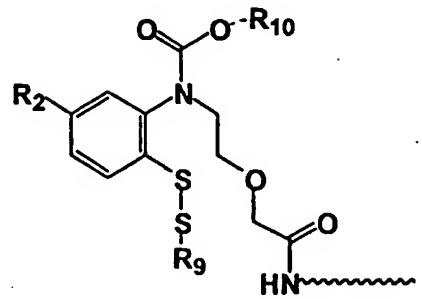
TR3



wherein Y is a N bearing group such as NH, R7NH where R7 is a lower alkyl group which may be substituted, or an –S- group, or an –O- group.; wherein R_1, R_2, R_3, and R_4 can be H, a halogen, Cl, F, Br, I, nitro, CH_3, a lower alkyl group, a lower alkoxy group, a sulphonate group, a phosphonate, a phosphate group, or -CO-R_8; where R_8 is OH, a lower alkoxy group, or where R_8 can be such that COR_8 comprises an amide and R_2-R_4 can also be an amino group, a substituted amino group; and HY is the compound released upon trigger activation; R_5 is a masked nucleophile, such as OH, SH, or NH_2, which is masked in a bioreversible fashion;. R_6 is H, or an alkyl group, which can bear inert substituents; wherein R_1-R_6 can have a site of linker attachment to the remainder of the drug complex.

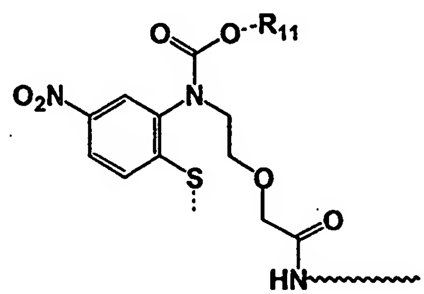
In a preferred embodiment, R_5 is a disulfide, R_2 is H, or nitro, Y is –O-, and R_6 is a linker for attachment to the remainder of the drug complex.

A preferred embodiment (embodiment TR4) of a trigger comprises the following structure:



TR4

wherein R_2 is H, or nitro; R_9 is any group such that the resulting S-S bond can be reduced by cells to give the corresponding thiol; R_9 can be an alkyl or aryl group, which can bear substituents; and R_9 can be a cysteine or a derivative of cysteine. Substituents on R_9 can include amino, hydroxy, phosphonate, phosphate, or sulfate, which can serve to increase water solubility. R_9 can also be a complex structure such that, both thiol groups that are generated from reduction of the disulfide, each trigger the release of independent drugs. R9 can be a group such as:

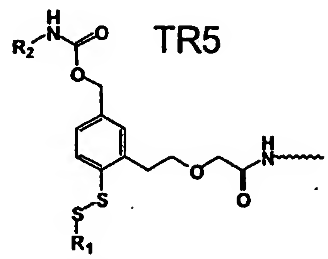


and wherein R_{10}-OH and R_{11}-OH are the compounds that are freed upon activation of the trigger; and wherein the wavy line is the site of attachment of the trigger to the remainder of the drug complex.

Triggers of this class function by a rapid cyclization reaction due to the high effective molarity of the neighboring nucleophile. The following references relate to this subject matter: Hutchins J.E.C.; Fife T.H., "Fast Intramolecular Nucleophilic Attack by Phenoxide Ion on Carbamate Ester Groups," *J Am Chem Soc,* 95(7):2282-2286 (1973); and Fife T.H., et al., "Highly Efficient Intramolecular Nucleophilic Reactions. The Cyclization of *p*-Nitrophenyl *N*-(2-Mercaptophenyl)-*N*-methylcarbamate and Phenyl *N*-(2-Aminophenyl)-*N*-methylcarbamate," *J Am Chem Soc,* 97(20):5878-5882 (1975), the contents of which are incorporated herein by reference in their entirety.

Triggers of this class provide a means of employing a hydroxy group on a drug as the site of trigger attachment, while producing a hydrolytically stable derivative. Triggers of this type can be activated principally inside cells since the concentration of glutathione is approximately 10-30 micromolar in plasma versus 1-10 mM inside cells. Thiol reductase activity is also chiefly intracellular. The following reference relates to this subject matter: Tew K.D., "Glutathione-associated Enzymes in Anti-cancer Drug Resistance," *Cancer Res,* 54:4313-4320 (1994), the contents of which is incorporated herein by reference in its entirety.

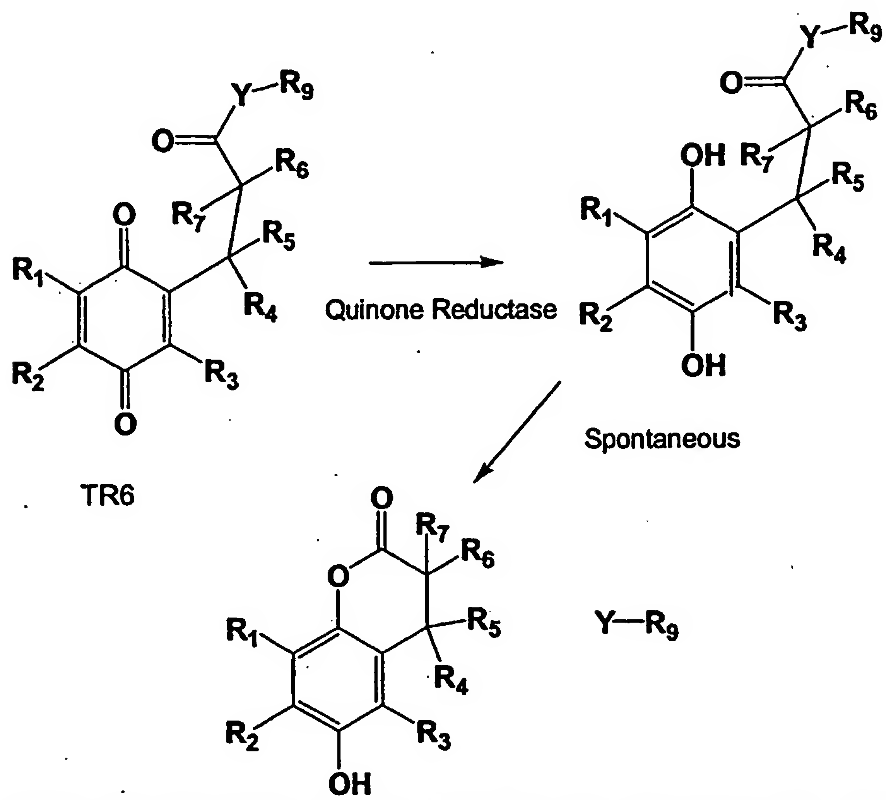
Another preferred embodiment (TR5) of an intracellular trigger, comprises the following structure:



TR5

wherein R_1 is a group such that the resulting S-S bond can be reduced by cells to give the corresponding thiol. R_1 can be a lower alkyl or aryl group, which can bear inert substituents. R_1 can be a cysteine or a derivative of cysteine. Substituents on R_1 can include: amino, hydroxy, phosphonate, phosphate, or sulfate groups that increase water solubility. R_1 can also be a complex structure such that both thiol groups, that are generated from reduction of the disulfide, each trigger the release of independent drugs; and wherein R_2-NH_2 is the drug or molecule that is freed upon activation of the trigger; and wherein the wavy line is the site of a linker attachment to the remainder of the drug complex.

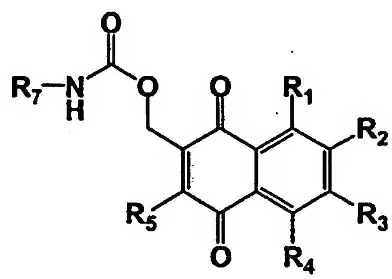
Another preferred embodiment (TR6)of an intracellular trigger comprises the structure shown below:



wherein Y is a N bearing group such as NH, or an –O- group, or an –S- group; and wherein HY-R_9 is the compound that is released upon activation of the trigger and $R_1 – R_7$ can be a hydrogen, alkyl groups, halogens, alkoxy, and -CO-R_8; where R_8 is OH; a lower alkoxy group, or where R_8 is such that COR_8 is an amide. Groups R_1-R_7 can bear a site for a linker attached to the site on the remainder of the drug. Triggers of this type are activated by quinone reductases, which function largely intracellularly. The up regulation of quinone reductase by tamoxifen in breast cancer cells is relevant to triggers of this type. The following references relate to this subject matter: Carpino LA, et al., "Reductive Lactonization of Strategically Methylated Quinone Propionic Acid Esters and Amides," *J Org Chem*, 54:3303-3310 (1989); and Montano, Monica M.;

Katzenellenbogen, Benita S., "The Quinone Reductase Gene: A Unique Estrogen Receptor-Regulated Gene that is Activated by Antiestrogens," *Proc Natl Acad Sci USA*, 94:2581-2586 (1997), the contents of which are incorporated herein by reference in their entirety.

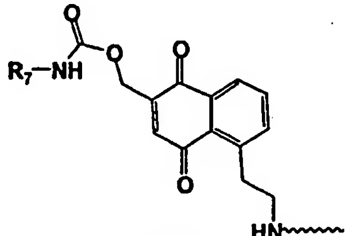
Another preferred embodiment (TR7) of intracellular triggers is comprised of the structure shown below:



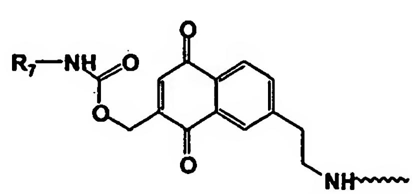
TR7

wherein R_1-R_5 can be H, methyl, ethyl, a lower alkyl group, methoxy, a lower alkoxy group, a halogen, Cl, Br, F, I, or -C(O)OR_8; where R_8 is a lower alkyl group, and wherein R_1-R_5 can also bear a site of linker attachment to the remainder of the drug complex; and wherein R_7-NH_2 is the compound liberated by trigger activation.

Preferred embodiments (TR8 and TR9) of triggers of this class are shown below:



TR8



TR9

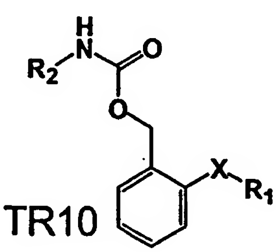
wherein the wavy line is the site of linker attachment. The triggers can be activated intracellularly either by quinone reductase or by nucleophilic addition of glutathione. The following reference relates to this subject matter: Flader C., et al., "Development of Novel Quinone Phosphorodiamidate Prodrugs Targeted to DT-Diaphorase," *J Med Chem,* 43:3157-3167 (2000), the contents of which is incorporated herein by reference in its entirety.

Clock-like Time Delayed Triggers

A common theme in multifunctional drug delivery function is localization of the drug to the tumor cells or target cells followed by the activation or unmasking of key components of the drug complex. The timing sequence is important. For example, premature unmasking of a nonspecific intracellular transport ligand can alter the pattern of drug targeting if the intracellular transport ligand has high affinity to its receptor. Clock-like time delayed triggers can allow the drug to have sufficient time to localize to the tumor before the consequences of trigger activation are manifested. The basis of clock-like triggers is that a triggering event initiates a spontaneous chemical reaction that proceeds with a predictable and suitable half-life.

In a preferred embodiment (TR10), the trigger comprises the following structure:

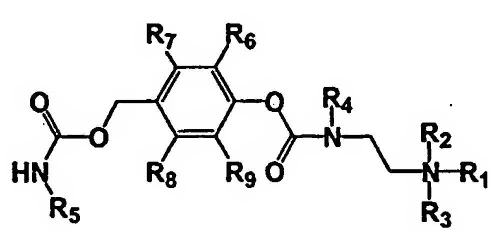
TR10



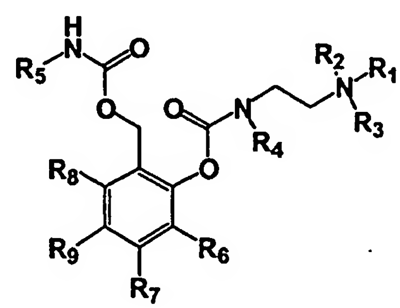
wherein X is O, NH, NCH_3, or S, and R_1 is a bioreversible protecting group which either spontaneously or by enzyme mediated processes is cleaved to unmask –OH, SH, or NH_2, and wherein NH_2-R_2 is the compound that is liberated upon trigger activation.

Ortho positioned electron donating groups promote elimination of benzylic compounds at rates that are slower than the corresponding para derivatives and provide for a time delay clock-like trigger. For example, under conditions in which para thio-benzyl carbamates undergo elimination with a half-life of 10 minutes the corresponding ortho derivative has a half-life of 72 min. Similar behavior is expected for ortho hydroxy, and ortho amino benzylic derivatives. The rate of solvolysis can be adjusted by placing electron-donating or electron withdrawing substituents on the benzylic ring. The following reference relates to this subject matter: Senter, Peter D., et al., "Development of a Drug-Release Strategy Based on the Reductive Fragmentation of Benzyl Carbamate Disulfides," *J Org Chem,* 55:2975-2978 (1990), the contents of which is incorporated herein by reference in its entirety.

Other preferred embodiments (TR11 and TR12) of a clock-like time delay trigger are comprised of the structures shown below:



TR11



TR12

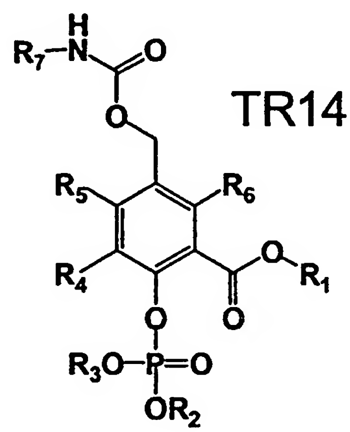
wherein R_1 is a bioreversible amino protecting group; R_2, R_3, and R_4 are H, methyl, ethyl, propyl, or a lower alkyl group; and R_6, R_7, R_8 and R_9, are H, a halogen, Cl, Br, I, F, methyl, ethyl, methoxy, or a lower alkoxy group; R_6 and R_9 can be a hydroxy group. Additionally, R_6, R_7, R_8, and R_9 can be the site of linker attachment to the remainder of ET complex; and wherein R_5-NH_2 is the compound that is liberated upon trigger activation.

Activation of these triggers by cleavage of the N-R_1 bond enhances the nucleophilicity of the amino group and initiates a spontaneous cyclization reaction leading to unmasking of the phenolic hydroxy group. The unmasked hydroxy group in turn triggers decomposition of the carbamate group. The half-life of the cyclization reaction can be varied by changing groups R_2- R_4 and R_6-R_9. Increasing steric bulk at R_2, R_3, and R_4 can slow the reaction. Substituents on the phenyl ring that are electron donating and increase the pKa of the corresponding phenol can slow the reaction.

In a preferred embodiment (TR13), R_2 and R_3 are methyl or ethyl and R1 is an acyl-oxy-methyl group or a phosphono-oxy-methyl group. The resulting positively charged ammonium group cannot participate as effectively in intramolecular catalysis of the carbamate decomposition. Cleavage of the acyl-oxy-methyl group by esterase or of the phosphono-oxy-methyl group by phosphatase can unmask a tertiary amino group. The tertiary amino group can then catalyze the hydrolysis of the carbamate by a cyclic intermediate with a half-life of approximately 40 minutes for the case in which R_2=R_3=R_4=methyl. The following references relate to this subject matter: Saari W.S., et al.,

"Cyclization-Activated Prodrugs. Basic Carbamates of 4-Hydroxyanisole," *J Med Chem,* 33:97-101 (1990); Krise J. P., et al., "Novel Prodrug Approach for Tertiary Amines: Synthesis and Preliminary Evaluation of *N*-Phosphonooxymethyl Prodrugs," *J Med Chem,* 42:3094-3100 (1999); and Krise J.P., et al., "A Novel Prodrug Approach for Tertiary Amines. 3. In Vivo Evaluation of Two *N*-Phosphonooxymethyl Prodrugs in Rats and Dogs," *J Pharm Sciences,* 88(9):928-932 (1999), the contents of which are incorporated herein by reference in their entirety.

Another preferred embodiment (TR14) of a clock-like time delay trigger is comprised of the following structure:



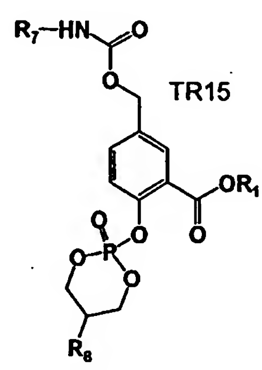
TR14

wherein R_1 is a group such that the resulting ester is cleaved either spontaneously or by esterases, and R_2 and R_3 are methyl, ethyl, or lower alkyl groups. R_2 and R_3 can be connected by one or more methylene groups, which can bear inert substituents; and wherein R_4 can be H, OH, methoxy, a lower alkoxy group, methyl, ethyl, or a halogen, or Cl, or F, or I, or Br; . R_5 and R_6 can be H, methoxy, a lower alkoxy group, methyl, ethyl, or Cl, Br, F, I, ; and wherein

R1-R_6 can also bear a site of linker attachment to the remainder of the drug complex; and wherein R_7-NH_2 is the compound liberated by trigger activation.

Triggers of this structure are activated by cleavage of the carboxylic acid ester. The carboxylate group then, by an intramolecular nucleophilic reaction, unmasks the phenolic hydroxy group that in turn initiates decomposition of the carbamate group. The half-life of the intramolecular nucleophilic reaction can be adjusted by varying the nature of the substituents R_2 and R_3. Increasing steric bulk can slow the reaction. Electron donating groups that increase the pKa of the phenolic OH group can also slow the reaction. Steric bulk at R_4 and R_5 can increase the rate. The following reference relates to this subject matter: Bromilow R.H., et al., "Intramolecular Catalysis of Phosphate Triester Hydrolysis. Nucleophilic Catalysis by the Neighbouring Carboxyl Group of the Hydrolysis of Dialkyl 2-Carboxyphenyl Phosphates," *J Chem Soc,* 1091-1096 (1971), the contents of which is incorporated herein by reference in its entirety.

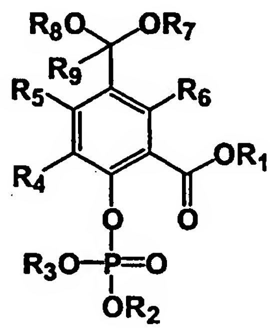
A preferred embodiment (TR15) of the above embodiment has the following structure:



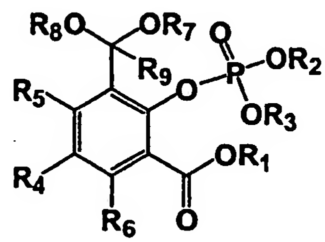
TR15

wherein R_8 is H, or O-P(O) $(OH)_2$. The intramolecular nucleophilic reaction is expected to proceed with a half-life of approximately 90 minutes under physiological conditions for compounds of the above structure with R_8=H.

Other preferred embodiments (TR16 and Tr17) of a clock-like time delay triggers are comprised of the structures shown below:



TR16

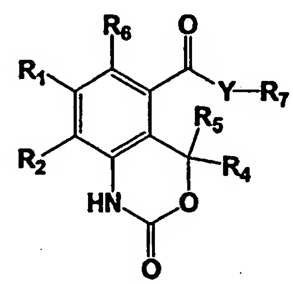


TR17

wherein R_1 is a group such that the resulting ester is cleaved either spontaneously or by esterases, and R_2 and R_3 are methyl, ethyl, or lower alkyl groups. R_2 and R_3 can be connected by one or more methylene groups which can bear substituents; and wherein R_4 can be H, OH, methoxy, a lower alkoxy group, methyl, ethyl, or Cl, Br, F, I, . R_5 and R_6 can be H, methoxy, a lower alkoxy group, methyl, ethyl, or Cl, Br, F, I, , and R_9 can be H, methyl, ethyl, or a lower alkyl group, and wherein R_1-R_6 and R_9 can also bear a site of linker attachment to the remainder of the drug complex; and wherein R_7-OH and R_8-OH are the compounds liberated by trigger activation. R_7 and R_8 can also be connected parts of a single compound, which is released upon trigger activation.

Triggers of this type can be activated by esterase. The resulting carboxylate group can by an intramolecular nucleophilic reaction with the phosphotriester group unmasking the phenolic hydroxy group. The phenolic hydroxy group in equilibrium with the phenolate ion can stabilize carbocation formation at the benzylic carbon and can trigger acetal decompostion.

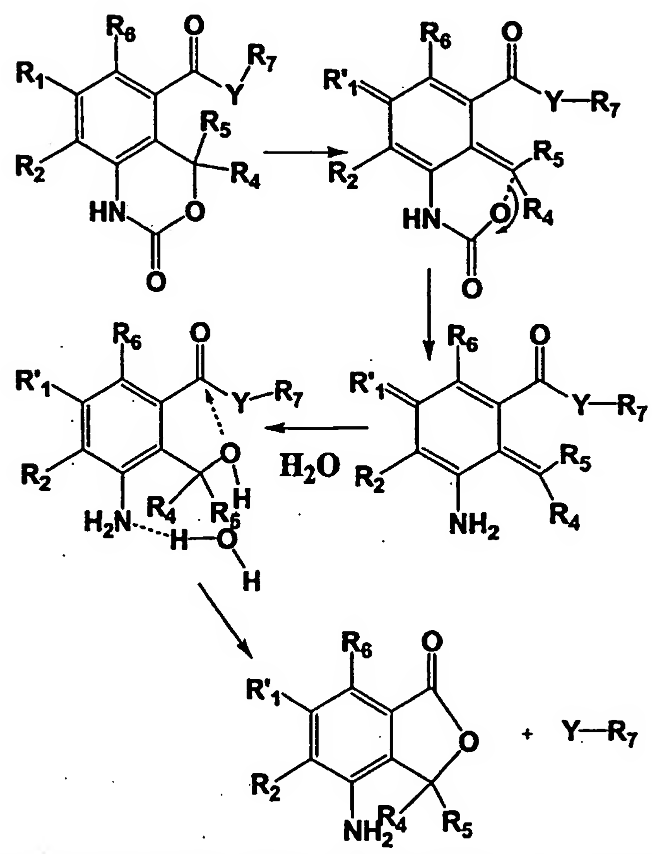
Another preferred embodiment (TR18) of a clock-like time delay trigger comprises the structure shown below:



TR18

Wherein Y is a N bearing group such as NH, or an –O- group, or an –S- group; and wherein Y-R_7 is the compound that is released upon activation of the trigger; and wherein R_2 and R_6 can be a wide range of groups including: hydrogen, alkyl groups, halogens, alkoxy, and -CO-R_8; where R_8 is OH; or a lower alkoxy group, or where R_8 is selected such that COR_8 is an amide. Groups R_4 and R_5 can be H, an alkyl group, or a phenyl group that can optionally be substituted. Group R_1 is a group of the type described in Table 1 that can undergo transformation to an electron donating group. Groups R_1-R_6 can optionally bear a site for a linker attached to a site on the remainder of the drug.

This trigger is activated by conversion of R_1 to an electron donating group that initiates cleavage of the benzylic C-O bond. Readdition of the carboxylate group to the benzylic carbon can compete with decarboxylation effectively slowing the rate of carbamate fragmentation as compared to that for noncyclic carbamates. The reactive quinone methide type intermediate can react with water forming a benzylic alcohol. The benzylic alcohol then can undergo intramolecular cyclization and cleave the carboxylate ester or amide functionality releasing Y. The rate can be increased by intramolecular base catalysis via the unmasked meta amino group. The mechansim is shown below:



The following reference relates to this subject matter: Fife, Thomas H. and Benjamin, Bruce M., "Intramolecular General Base Catalyzed Alcoholysis of

Amides," *J Chem Soc Chem Comm*, 14:525-527 (1974), the contents of which is incorporated herein by reference in its entirety.

Triggers can also be strategically placed in groups such as esters, amides, disulfides, acetals, carbonates, and enol ethers which undergo spontaneous or enzymatic transformation and which initiates the intended change in drug function upon activation or in vivo modification.

Detoxification Triggers

Triggers can effect toxification or detoxification of the drug depending upon the particular design. Detoxification triggers can function in a variety of manners. The trigger can impair function of the drug directly or can impair intracellular transport of the drug. For a discussion of detoxification triggers see the following references that relate to this subject matter: 5,274,162, 12/28/93, Glazier A., "Antineoplastic Drugs with Bipolar Toxification/Detoxification Functionalities"; and 5,659,061, 8/19/97, GlazierA., "Tumor Protease Activated Prodrugs of Phosphoramide Mustard Analogs with Toxification and Detoxification Functionalities", the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment of the present invention the detoxification trigger functionally detoxifies the drug by uncoupling the drug from the targeting intracellular transport functionalities. In one embodiment the detoxifier trigger cleaves the active drug coupled to a linker which functions to prevent non-

selective cellular uptake. Ionic compounds diffuse very poorly through cell membranes and can be employed in the linker for this purpose.

The factors that trigger detoxification can be specific or non-selective. As discussed previously, a preferred embodiment consists of a drug in which the detoxification trigger can be activated by an enzyme that is selectively delivered to vital non-target cells such as bone marrow stem cells. In a preferred embodiment a detoxification trigger can be activated preferentially in non-target locations. A wide range of detoxifying triggers can be used with the approach of targeted delivery of the detoxifying enzyme to non-tumor cells. Considerations for a selectively targeted detoxification enzyme are as follows:

1.) The enzyme activity deliverable to non-tumor cells can be sufficient to effect detoxification;
2.) The enzyme preferably should be of low toxicity to the normal cells;
3.) The enzyme preferably should not stimulate an autoimmune response against the normal cells;
4.) The affinity of the detoxifying enzyme for the detoxifying trigger preferably should be lower than the affinity of the drug to the targeted tumor cells;
5.) The targeted enzyme preferably should be retained by the cells on the cell surface and not be rapidly internalized;
6.) The level of detoxifying enzyme activity present in the microenvironment of the tumor cells preferably should be insufficient to impede the delivery of a cytotoxic effect to the tumor cells; and
7.) The enzyme preferably should not be rapidly inhibited by plasma factors.

In a preferred embodiment of the invention the detoxification trigger can be a substrate for aryl sulfatase. Aryl sulfatase activity of the blood is low. For example, bone marrow stem cells are characterized by the presence of the CD34 antigen on the cell membrane. A complex of human aryl sulfatase linked to a humanized monoclonal antibody specific for CD34 could be employed to selectively deliver a detoxifying quantity of aryl sulfatase to protect vital bone marrow stem cells (provided, of course the malignancy is CD34 negative). The following references relate to this subject matter: Civin C.I., et al., "Sustained, Retransplantable, Multilineage Engraftment of Highly Purified Adult Human Bone Marrow Stem Cells In Vivo," *Blood*, 88(11): 4102-9 (1996); Hill B., et al., "High-Level Expression of a Novel Epitope of CD59 Identifies a Subset of CD34+ Bone Marrow Cells Highly Enriched for Pluripotent Stem Cells," *Exp Hematol*, 24(8):936-43 (1996); Civin C.I., et al., "Highly Purified CD34-Positive Cells Reconstitute Hematopoiesis," *J Clin Oncol*, 14(8): 2224-33 (1996); and Civin C.I., et al., "Purification and Expansion of Human Hematopoietic Stem/Progenitor Cells," *Ann NY Acad Sci*, 770:91-8 (1995), the contents of which are incorporated herein by reference in their entirety.

The use of a human enzyme and humanized monoclonal antibodies can allow for multiple cycles of therapy without problems related to allergenicity. It is also worth emphazing that the presence of circulating anti-CD34 antibody-aryl sulphatase molecules is not expected to significantly interfere with drug delivery to the targeted tumor cells. The reason for this is that the plasma concentration of the drug can be orders of magnitude lower than the Km for the aryl sulfatase

towards the detoxifying trigger. This can be compensated for on bone marrow stem cells by the high enzyme concentration at the membrane surface.

Another application of a detoxification trigger is to serve as a time clock. The detoxification trigger can be selected to be activated by nonspecific mechanisms and to initiate detoxification at a predictable rate. This provides the functional equivalent of exposing cells to a timed pulse of active drug. The quantity of drug that the target cells internalize during that time pulse is a function of the rate of uptake. The rate of uptake is a different parameter than the quantity of drug bound to the cells. For example, if tumor cells internalize the drug much faster due to the cross linking of receptors it can be useful to employ such a detoxification trigger, which serves as a time clock.

The combination of a time clock-like detoxification trigger and a tumor-selective trigger for the masked transport ligand provides unique opportunities to refine targeting specificty. One can combine the selectivity of the targeting mechanism of the drug E-T with the targeting specificity of the tumor-selective activating antibody-enzyme complex to achieve enhanced degrees of selectivity. This embodiment of the present invention consists of:

1.) Selecting the masked transport ligand trigger to be such that it is specifically activated by an enzyme referred to as "EZ";

2.) Selectively delivering EZ to the tumor cells via a tumor antigen specific antibody-EZ complex (or functional analog thereof); or other target agent;

3.) Selecting the detoxification trigger to be activated by a nonspecific clock-like mechanism which provides a sufficient half-life for target cell

associated drug to be toxified by the target cell associated antibody-EZ and internalized; and

4.) Selecting the targeting ligands of the drug to be specific for target cell associated receptors that are inefficiently internalized so that intracellular transport is dependent on the intracellular transport ligand.

Since the ultimate target specificity is defined by both the targeted drug and the targeted activating enzyme neither need to have extraordinary tumor selectivity in order to achieve precision targeting. The role of the detoxification trigger is to provide a time limit to the process and restrict toxicity to those sites with efficient cellular uptake, which can correspond to targeted cells.

Masking Triggers

An important application of triggers is to allow a chemical group of the drug complex to be masked or hidden like a trojan horse until the trigger is activated. Numerous examples are given in other sections. The function of masking triggers is to prevent the modified or masked group of exerting its biological activity until trigger activation. A masking trigger is comprised of a chemical structure covalently coupled to a compound which prevents the binding of that compound to a receptor and wherein activation of the masking trigger by spontaneous or enzymatic processes cleaves the bond or bonds between the masking trigger and the compound thereby restoring receptor binding activity.

Intracellular Trapping Ligands

For most anti-cancer drugs the intracellular concentration is the key determinant of cytotoxicity. This is a function of the rate of drug influx and drug efflux. Many tumors exhibit drug resistance by actively pumping anti-cancer drugs out of the cells. To counteract this outward drug flux current drugs are often given in high doses. However, targeted anti-cancer drugs preferably can be given at ultra-low doses such that the concentration outside the cells is close to zero. Under these conditions diffusion of the drug out of the target cell is favored. The function of intracellular trapping ligands is to prevent drug that is intracellular from escaping to outside the cell.

Intracellular trapping ligands can be tumor-selective or non-selective. If the intracellular trapping ligand binds to a receptor that is selectively enriched in tumor cells, enhance tumor targeting selectivity can result. The intracellular trapping ligand can also function to target the drug to critical intracellular locations such as the nucleus or mitochondria and thereby enhance drug activity. The interaction between the intracellular trapping ligand its receptor can be irreversible or reversible, but with high affinity. A wide range of groups that can be adapted for use as intracellular trapping ligands are described in the neoantigen section of this application.

An intracellular trapping ligand is comprised of a group which has the following properties:

1.) The ligand is able to bind with sufficient and preferably high affinity or irreversibly to one or more intracellular receptors (intracellular structures or components);

2.) The ligand must have a site to which a linker can be attached that does not interfere with receptor binding; and

3.) If the intracellular trapping ligand has significant affinity to extracellular structures than it is preferable to employ a masked intracellular trapping ligand.

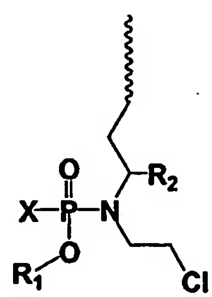
A masked intracellular trapping ligand is comprised of an intracellular trapping ligand a masking trigger that is preferentially activated inside cells; such that the masking trigger inactivates or interferes with the ability of the group to bind to its receptor and wherein activation of the intracellular trigger can unmask the functional intracellular trapping ligand. Intracellular triggers described in the trigger section of this document can be used as components of masked intracellular trapping ligands.

Non-selective Intracellular Trapping Ligands

In a preferred embodiment, the intracellular trapping ligand is comprised of a masked functionality, which is able to covalently bind to cellular structures following activation of a trigger. The mechanisms of covalent modification of cellular proteins compatible with this embodiment of the invention include: the reaction of electrophiles with nucleophilic groups such as thiols, amines, and hydroxy groups of the proteins; the reaction of nucleophiles with electrophilic

centers in proteins and free radical reactions. It is preferrable to employ groups, which require triggering to unmask the reactivity required for protein modification. This can allow the drug targeting specificity to be defined by the high affinity interaction of the targeting ligands the target receptors rather than by the pattern of nonspecific covalent protein modification. The use of chemically stable drugs, which require triggering to unmask reactivity, also has major practical pharmaceutical advantages. A large number of compounds are known, which require triggering or bioactivation for the unmasking of the chemical reactivity including: phosphoramide mustard analogs, quinone methide precursors, enediynes, and nitroimadazoles.

A preferred embodiment (embodiment IT1) of a non-selective intracellular trapping ligand is comprised of the structure shown below:

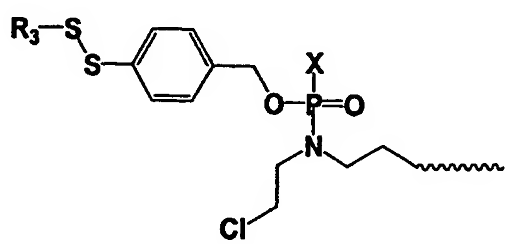


wherein X is NH_2, CH_3NH, $(CH_3)_2N$, CH_3, C_6C_5, $C_6H_5CH_2$, a substituted benzyl or a substituted phenyl group, CH_3O, or a lower alkoxy group, and the wavy line is the site of linker attachment to the toxin group, and wherein R1 is a protecting group which when triggered results in umasking of the free hydroxy group on the phosphorous. R_1 can also bear a site for linker attachment to the remainder of the targeted drug. In this case, the structure R_1 above can serve a dual function

of both freeing the toxin – intracellular trapping ligand from the remainder of the targeted drug complex and activating it towards nucleophilic attack. R_2 is H or CH_2CH_2Cl.

A large number of suitable embodiments of the group R_1 are described in the section on triggers and in 5,627,165, 5/06/97 Glazier A., "Phosphorous Prodrugs and Therapeutic Delivery Systems Using Same". Unmasking of the free OH group on the phosphorous can dramatically increase reactivity towards nucleophiles on adjacent proteins. The conversion of the phosphoester to the negatively charged species enormously increases the nucleophilicity of the adjacent nitrogen and triggers the formation of a highly reactive aziridinium cation, which can rapidly alkylate nucleophiles.

Another preferred embodiment (embodiment IT2) of an intracellular trapping ligand is shown below:



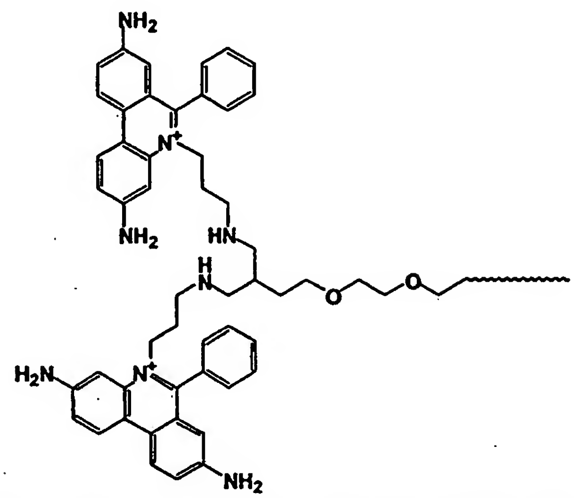
wherein R_3 is a group such that the resulting disulfide can be cleaved intracellularly. In preferred embodiments, R_2-SH is cysteine, an oligopepetide

containing cysteine, or an analog of cysteine in which the amino and or carboxylate groups are derivatized.

Intracellular Trapping Ligands Selective for DNA

The site of action of many anti-cancer drugs is on cellular DNA and on nuclear enzymes. In a preferred embodiment, the intracellular targeting ligand is a group that binds to DNA. A large number of agents that bind to DNA are known and can serve the dual function of trapping the drug intracellularly and focusing the drug to site of action at DNA. A preferred embodiment comprised of ethidium homodimer, which binds with high affinity to DNA. The following references relate to this subject matter: Gaugain B., et al., "DNA Bifunctional Intercalators. I. Synthesis and Conformational Properties of an Ethidium Homodimer and of an Acridine Ethidium Heterodimer," *Biochemistry,* 17(24):5071-8 (1978); Gaugain B., et al., "DNA Bifunctional Intercalators. 2. Fluorescence Properties and DNA Binding Interaction of an Ethidium Homodimer and an Acridine Ethidium Heterodimer," *Biochemistry,* 17(24):5078-88 (1978); Markovits J., et al., "Ethidium Dimer: A New Reagent for the Fluorimetric Determination of Nucleic Acids," *Anal Biochem,* 94(2):259-64 (1979); Glazer A.N., et al., "A Stable Double-Stranded DNA-Ethidium Homodimer Complex: Application to Picogram Fluorescence Detection of DNA in Agarose Gels," *Proc Natl Acad Sci,* 87:3851-3855 (1990), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment (embodiment IT3), the intracellular trapping ligand comprises the following structure:



wherein the wavy line is the site of linker attachment to the toxin moiety.

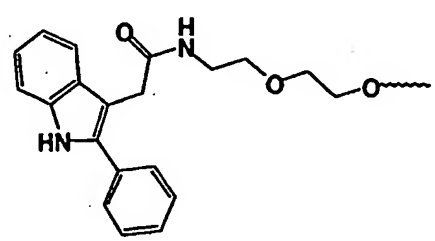
Intracellular Trapping Ligands Selective for Mitochondria

Mitochondria are an important site of action of many anti-cancer drugs. In a preferred embodiment, the intracellular trapping ligand is a group that binds to mitochondrial components. The peripheral benzodiazepam receptor (PBR) is a protein that is localized on the outer mitochondrial membrane and microsomal membranes. Although PBR is widely distributed it is enriched in a variety of tumors. A number of compounds that bind with nanomolar to subnanomolar affinity to PBR are known.. The following references relate to this subject matter: Trapani G., et al., "Synthesis and Binding Affinity of 2-Phenylimidazo[1,2-Alpha]Pyridine Derivatives for Both Central and Peripheral Benzodiazepine Receptors. A New Series of High-Affinity and Selective Ligands for the Peripheral Type," *J Med Chem,* 40(19):3109-18 (1997); Campiani G., et al.,

"Synthesis, Biological Activity, and SARs of Pyrrolobenzoxazepine Derivatives, a New Class of Specific "Peripheral-Type" Benzodiazepine Receptor Ligands," *J Med Chem,* 39(18):3435-50 (1996); Chaki S., et al., "Binding Characteristics of [3H]DAA1106, a Novel and Selective Ligand for Peripheral Benzodiazepine Receptors," *Eur J Pharmacol,* 371(2-3):197-204 (1999); Dussossoy D., et al., "Development of a Monoclonal Antibody to Immuno-Cytochemical Analysis of the Cellular Localization of the Peripheral Benzodiazepine Receptor," *Cytometry,* 24(1):39-48 (1996); Batra S.; Iosif C.S., "Elevated Concentrations of Mitochondrial Peripheral Benzodiazepine Receptors in Ovarian Tumors," *Int J Oncol,* 12(6):1295-8 (1998); Beinlich A., et al., "Specific Binding of Benzodiazepines to Human Breast Cancer Cell Lines," *Life Sci,* 65(20):2099-108 (1999); Venturini I, et al., "Increased Expression of Peripheral Benzodiazepine Receptors and Diazepam Binding Inhibitor in Human Tumors Sited in the Liver," *Life Sci,* 65(21):2223-31 (1999); Taketani S., et al., "Involvement of Peripheral-Type Benzodiazepine Receptors in the Intracellular Transport of Heme and Porphyrins," *J Biochem (Tokyo),* 117(4):875-80 (1995); Davies L.P., et al., "New Imidazo[1,2-B]Pyridazine Ligands for Peripheral-Type Benzodiazepine Receptors on Mitochondria and Monocytes," *Life Sci,* 57(25):PL381-6 (1995); Trapani G., et al., "Novel 2-Phenylimidazo[1,2-A]Pyridine Derivatives as Potent and Selective Ligands for Peripheral Benzodiazepine Receptors: Synthesis, Binding Affinity, and in Vivo Studies," *J Med Chem,* 42(19):3934-41 (1999); Bono F., et al., "Peripheral Benzodiazepine Receptor Agonists Exhibit Potent Antiapoptotic Activities," *Biochem Biophys Res Commun,* 265(2):457-61 (1999); Batra S.; Iosif C.S., "Peripheral Benzodiazepine Receptor in Human Endometrium and Endometrial Carcinoma,"

Anti-cancer Res, 29(1A):463-6 (2000); Beinlich A., et al., "Relation of Cell Proliferation to Expression of Peripheral Benzodiazepine Receptors in Human Breast Cancer Cell Lines," *Biochem Pharmacol,* 60(3):397-402 (2000); Hardwick M., et al., "Peripheral-Type Benzodiazepine Receptor (PBR) in Human Breast Cancer: Correlation of Breast Cancer Cell Aggressive Phenotype with PBR Expression, Nuclear Localization, and PBR-Mediated Cell Proliferation and Nuclear Transport of Cholesterol," *Cancer Res,* 59(4):831-42 (1999); Alenfall J., et al., "Cytotoxic Effects of 125I-Labeled PBZr Ligand PK 11195 In Prostatic Tumor Cells: Therapeutic Implications," *Cancer Lett,* 134(2):187-92 (1998); Venturini I., et al., "Up-Regulation of Peripheral Benzodiazepine Receptor System in Hepatocellular Carcinoma," *Life Sci,* 63(14):1269-80 (1998); Kozikowski A.P., et al., "Synthesis and Biology of a 7-Nitro-2,1,3-Benzoxadiazol-4-Yl Derivative of 2-Phenylindole-3-Acetamide: A Fluorescent Probe for the Peripheral-Type Benzodiazepine Receptor," *J Med Chem,* 40(16):2435-9 (1997), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment (embodiment IT4), the intracellular trapping ligand is a group that binds to PBR. In a preferred embodiment, the group comprises the following structure:

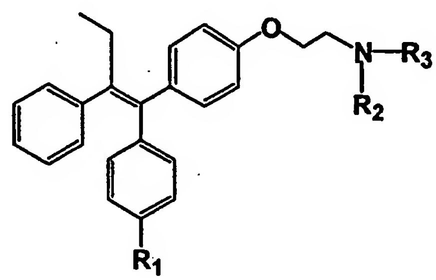


wherein the wavy line is the site of linker attachment to the toxin.

Intracellular Trapping Ligands Selective for Estrogen Receptors

Estrogen receptors (ER) are over-expressed in a number of important human malignancies and can be employed to both trap drugs inside cells and to deliver the drug to the cell nucleus. (See the neoantigen section for a discussion on estrogen receptors and related references.) In a preferred embodiment, the intracellular trapping ligand comprises a group, that binds to estrogen receptors.

In preferred embodiments (embodiments IT5), the intracellular trapping ligand comprises the following structure based on tamoxifen:



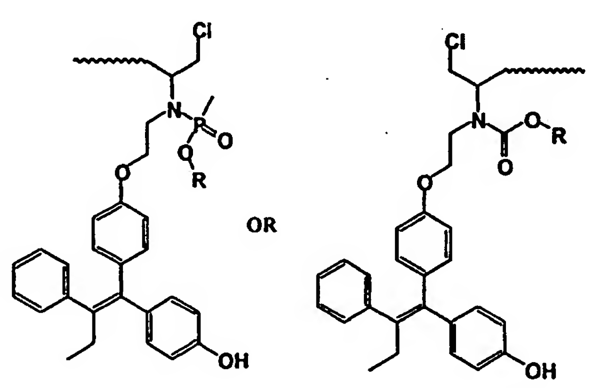
wherein R_1 is H, or OH, or the site of attachment of a trigger connected to the remainder of the targeted drug such that activation of the trigger liberates the tamoxifen analog; and wherein R_2 is H or methyl, and R_3 is the site of linker attachment to the toxin moiety of the drug.

Other preferred embodiments are based on the ability of tamoxifen aziridine and related compounds to irreversibly bind to ER by alkylation of a cysteine residue. The following references relate to this subject matter: Katzenellenbogen J.A., et al., "Efficient and Highly Selective Covalent Labeling of the Estrogen Receptor

with [^{3}H]Tamoxifen Aziridine," *J Biol Chem,* 258(6):3487-3495 (1983); Harlow K.W., et al., "Identification of Cysteine 530 as the Covalent Attachment Site of an Affinity-labeling Estrogen (Ketononestrol Aziridine) and Antiestrogen (Tamoxifen Aziridine) in the Human Estrogen Receptor," *J Biol Chem,* 264(29):17476-17485 (1989); Reese J.C.; Katzenellenbogen B.S., "Mutagenesis of Cysteines in the Hormone Binding Domain of the Human Estrogen Receptor," 266(17):10880-10887 (1991); Aliau S., et al., "Cysteine 530 of the Human Estrogen Receptor α is the Main Covalent Attachment Site of 11β-(Aziridinylalkoxyphenyl)estradiols," *Biochemistry,* 38:14752-14762 (1999), the contents of which are incorporated herein by reference in their entirety.

In these embodiments the intracellular trapping ligand is comprised of an ER binding ligand to which is coupled a latent alkylating agent which is unmasked upon activation of a trigger.

In a preferred embodiment (embodiment IT6 and IT7), the intracellular trapping ligand comprises the following structure:

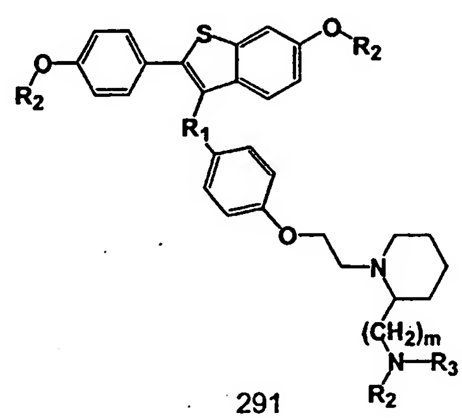


IT6 OR IT7

wherein R is a trigger attached to the remainder of the targeted drug such that activation of the trigger cleaves the phophoester or carbamate generating an electrophilic species and wherein the wavy line is the site of linker attachment to the toxin group. A wide variety of suitable triggers have been described elsewhere in this patent. The trigger group R can also bear a site of attachment to the remainder of the targeted drug complex in which case activation of the trigger serves the dual function of both freeing the toxin – intracellular trapping ligand from the remainder of the targeted drug complex and activating it towards nucleophilic attack.

Another preferred set of structures is based on raloxifene. The following references relate to this subject matter: Palkowitz A.D., et al., "Discovery and Synthesis of [6-Hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]- 2-(4-hydroxyphenyl)]benzo[*b*]thiophene: A Novel, Highly Potent, Selective Estrogen Receptor Modulator," *J Med Chem*, 40(10):1407-1416 (1997), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiments (embodiments IT8) of an intracellular trapping ligands is comprised of the structure shown below:

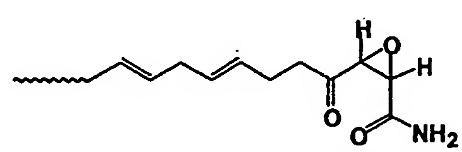


wherein R_1 is CO, CH_2, S, O, or NH, and m = 1 to 6; R_2 is H, or the site of attachment of a trigger connected to the remainder of the targeted drug such that activation of the trigger liberates the raloxifene analog, and R_3 is the site of linker attachment to the toxin group.

Intracellular Trapping Ligands Selective for Fatty Acid Synthase

In a preferred embodiment, the intracellular trapping ligand is a mechanism based enzyme inhibitor of fatty acid synthase, an enzyme that is over-expressed in breast cancer, colon cancer, ovarian, endometrial and prostate cancer. (See the Neoantigen section for discussion and references.)

A preferred embodiment (embodiment IT9) comprises the following structure:



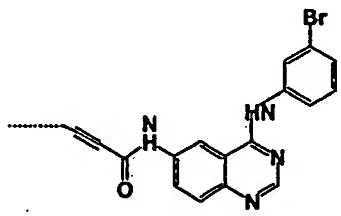
wherein the site of linker attachment to the rest of the drug is indicated by the wavy line.

Intracellular Trapping Ligands Selective for Epidermal Growth Factor Receptor

Epidermal growth factor receptors (EGFR) are membrane associated tyrosine kinases that are over-expressed in a large number of malignancies including: breast, prostate, ovarian, lung, gastric, and bladder. In a preferred embodiment,

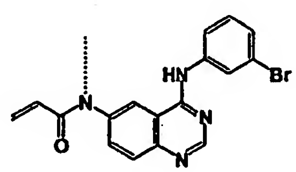
the intracellular trapping ligand is an irreversible inhibitor to EGFR (and members of the epidermal growth factor receptor family of proteins), that covalently modifies the protein. (See the Neoantigen section for discussion and references.)

In preferred embodiments (embodiments IT10), the intracellular trapping ligand comprises the following structures:

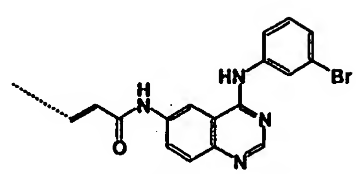


wherein the dotted line is the site of linker attachment to the remainder of the drug.

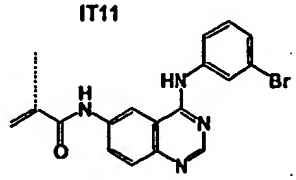
In other preferred embodiments (embodiment IT11, IT12, IT13, IT14, IT15, IT16, IT17, IT18, IT19, IT20 and IT21), E comprises the following structure:



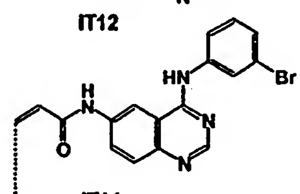
IT11



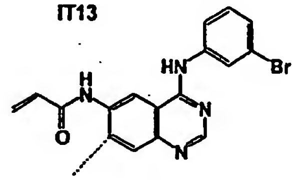
IT12



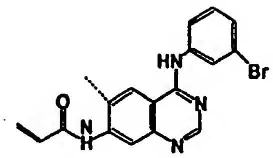
IT13



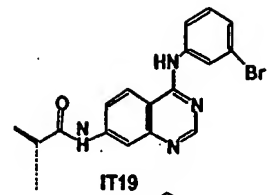
IT14



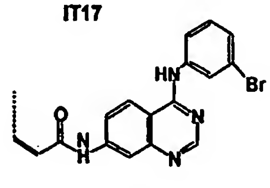
IT15



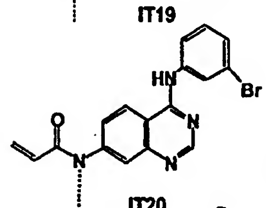
IT17



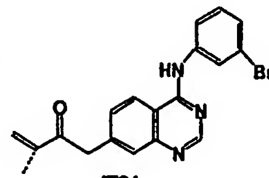
IT19



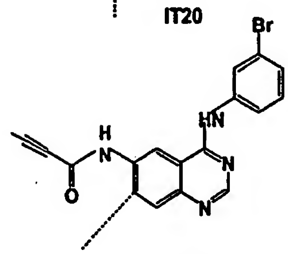
18



IT20



IT21



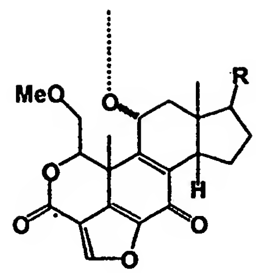
IT16

wherein the dotted line is the site of linker attachment to the remainder of the drug.

Intracellular Trapping Ligands Selective for Phospatidylinositol 3-Kinase

Phospatidylinositol 3-kinase (PIK3) is over-expressed in numerous malignancies including ovarian, breast, prostate, and lung cancer. In a preferred embodiment, the intracellular trapping ligand is an irreversible inhibitor of PIK3. (See the Neoantigen section for discussion and references.)

A preferred embodiment (emodiment IT22) comprises the following structure:



wherein the dotted line is the site of linker attachment to the remainder of the drug and R is O, or OH.

Effector Mechanisms and Effector Agents

Diagnostic Applications:

The present invention, E-T, can be employed with an enormous range of effector functionalities E, depending on the intended drug indication.

For diagnostic purposes, a wide E can be comprised of a wide range of entities that allow for detection using imaging techniques commonly employed in radiology and nuclear medicine. The following reference relates to this subject matter:; Reichert D.E., et al., "Metal Complexes as Diagnostic Tools," *Coordination Chemistry Reviews,* 184:3-66 (1999); the contents of which is hereby incorporated by reference in its entirety.

Examples include, radioactive moieties, ligands which bind radioisotopes, groups applicable to positron emission tomography, and groups applicable to magnetic resonance imaging, such as gadolinium chelates. The detector group

can also be a fluorescent moiety or a group such as biotin, which is amenable to histochemical detection for the applications related to histopathology.

Therapeutic Applications

Although the principle application of this invention is in the area of anti-cancer therapy, the invention can, in principle, be applied to many other areas of drug delivery. For example, the targeting methodology can be used to deliver a cytotoxic agent to a selected class of lymphocytes for the treatment of an autoimmune disease such as scleroderma or lupus erythematosis. The targeting technology can also be used to deliver any therapeutically useful enzyme, protein, or polynucleotide or oligonucleotide.

Anti-cancer Agents

A wide range of anti-cancer drugs can be selectively targeted to tumor cells with the present invention. The high target affinity of the drug E-T for tumor cells can potentially allow a reduction in the total drug dose employed by a factor of 1000 to perhaps 1 million fold compared to non-targeted drug. At these low doses toxicity of the non-targeted drugs generated by metabolism of the targeted drug can be completely inconsequential. However, the targeted drug complex can and potentilly has toxicity defined by the domain of targeting which can never be absolutely specific for tumor cells. Proper selection of the targeted drugs can influence the ultimate therapeutic index as much as selection of the target sites. The optimal situation is when the anti-cancer agent employed has some selective toxicity for tumor cells independent of targeting. Agents which are selectively directed against the mechanisms of cell replication are preferred.

Agents which, mediate toxicity by a single high affinity interaction with a single key enzyme are preferred over drugs with multiple mechanisms of action. For example, if a targeted drug E-T has some affinity for receptors in the heart and the drug delivered is adriamycin (a known cardiotoxin) then cardiotoxicty can result. On the other hand, if the drug delivered is a very selective inhibitor to thymidylate synthetase, (an enzyme nonessential to the heart) then cardiotoxicity is unlikely.

Toxins directed specifically against the key enzymes of cell replication are preferred. These include inhibitors to: thymidylate synthase, DNA polymerase alpha, Toposisomerase I and II, ribonucleotide reductase, Thymidylate kinase, cyclin dependent kinases, DNA primase, DNA helicase, and microtubule function.

Highly preferred embodiments of the invention are with E being comprised of two different anti-cancer drugs or an anti-cancer drug and an inhibitor to p-glycoprotein. Also included within the scope of the present invention is the embodiment in which E is comprised of one or more inhibitors to multi-drug resistance without a coupled toxin. The following references relate to this subject matter: Gottesman Michael M., "How Cancer Cells Evade Chemotherapy" Sixteenth Richard and Hinda Rosenthal Foundation Award Lecture", *Cancer Research*, 53:747-754 (1993); Roe M., et al., "Reversal of P-Glycoprotein Mediated Multi-drug Resistance by Novel Anthranilamide Derivatives," *Bioorg Med Chem Lett*, 9(4):595-600 (1999); Szakacs G., et al., "Diagnostics of Multi-drug Resistance in Cancer," *Pathol Oncol Res*, 4(4):251-7 (1998); Sumizawa T.,

et al., "Reversal of Multi-drug Resistance-Associated Protein-Mediated Drug Resistance by the Pyridine Analog PAK-104P," *Mol Pharmacol*, 51(3):399-405 (1997); Caner U., "Full Blockade of Intestinal P-Glycoprotein and Extensive Inhibition of Blood-Brain Barrier P-Glycoprotein by Oral Treatment of Mice with PSC833," *J Clin Invest*, 10(10):2430-6 (1997); Alexander D., "Histopathological Assessment of Multi-drug Resistance in Gastric Cancer: Expression of P-Glycoprotein, Multi-drug Resistance-Associated Protein, and Lung-Resistance Protein," *Surg Today*, 29(5):401-6 (1999); Zhou D.C., et al., "Effect of the Multi-drug Inhibitor GG918 on Drug Sensitivity of Human Leukemic Cells," *Leukemia*, 11(9):1516-22 (1997); Courtois A., et al., " Inhibition of Multi-drug Resistance-Associated Protein (MRP) Activity by Rifampicin in Human Multi-drug-Resistant Lung Tumor Cells," *Cancer Lett*, 139(1):97-104 (1999); Rappa G., et al., "New Insights into the Biology and Pharmacology of the Multi-drug Resistance Protein (MRP) from Gene Knockout Models," *Biochem Pharmacol*, 58(4):557-62 (1999); Kaye S.B., "Multi-drug Resistance: Clinical Relevance in Solid Tumours and Strategies for Circumvention," *Curr Opin Oncol*, 10 Suppl 1:S15-9 (1998); Mendez-Vidal C.; Quesada A.R., "Reversal of P-Glycoprotein-Mediated Multi-drug Resistance In Vitro by AV200, a New Ardeemin Derivative," *Cancer Lett*, 132(1-2):45-50 (1998); Atadja P., et al., "PSC-833, a Frontier in Modulation of P-Glycoprotein Mediated Multi-drug Resistance," *Cancer Metastasis Rev*, 17(2):163-8 (1998); Klopman G., et al., "Quantitative Structure-Activity Relationship of Multi-drug Resistance Reversal Agents," *Mol Pharmacol*, 52(2):323-34 (1997); Rabindran S.K., et al., "Reversal of a Novel Multi-drug Resistance Mechanism in Human Colon Carcinoma Cells by Fumitremorgin C," *Cancer Res*, 58(24):5850-8 (1998); Dale I.L., et al., "Reversal of P-Glycoprotein-

Mediated Multi-drug Resistance by XR9051, a Novel Diketopiperazine Derivative," *Br J Cancer*, 78(7):885-92 (1998); Wallstab A., et al., "Selective Inhibition of MDR1 P-Glycoprotein-Mediated Transport by the Acridone Carboxamide Derivative GG918," *Br J Cancer*, 79(7-8):1053-60 (1999); Chen G.; Waxman D.J., " Complete Reversal by Thaliblastine of 490-Fold Adriamycin Resistance in Multi-drug-Resistant (MDR) Human Breast Cancer Cells. Evidence that Multiple Biochemical Changes in MDR Cells Need not Correspond to Multiple Functional Determinants tor Drug Resistance," *J Pharmacol Exp Ther*, 274(3):1271-7 (1995); Mistry P., et al., "In vivo Efficacy of XR9051, a Potent Modulator of P-Glycoprotein Mediated Multi-drug Resistance," *Br J Cancer*, 79(11-12):1672-8 (1999), the contents of which are incorporated herein by reference in their entirety.

Preferred toxins include: anthracyclines, ellipticines, taxols, mitoxantrones, epothilones, quinazoline inhibitors of thymidylate synthase, stautosporin, podophyllotoxins, bleomycin, aphidicolin, cryptophycin-52, mitomycin c, phosphoramide mustard analogs, vincristine, vinblastine, and indanocine, and compounds with cytotoxicity for cells in the submicromolar range that are currently listed in the U.S. National Cancer Institute's Developmental Therapeutics Program"s, Human Tumor Cell Line Screen for Anti-cancer Agents data base which is accessible at http://dtp.nci.nih.gov/. The following references relate to this subject matter: Bisagni E., et al., "Synthesis of 1-Substituted Ellipticines by a New Route to Pyrido[4,3-*b*]-Carbazoles," *JCS Perkin I*, 8(1347):1706-1711 (1978); Martinez E.J., et al., "Phthalascidin, A Synthetic Antitumor Agent with Potency and Mode of Action Comparable to Ecteinascidin

743," *Proc Natl Acad Sci USA*, 96:3496-3501 (1999); Dongfang M., et al., "Remote Effects in Macrolide Formation Through Ring-Forming Olefin Metathesis: An Application to the Synthesis of Fully Active Epothilone Congeners," *J Am Chem Soc*, 119:2733-2734 (1997); Dai-Shi S., et al., "Structure-Activity Relationships of the Epothilones and the First In Vivo Comparison with Paclitaxel," *Angew Chem Int Ed Engl*, 36(19):2093-2096 (1997); Chou Ting-Chao, et al., "Desoxyepothilone B: An Efficacious Microtubule-Targeted Antitumor Agent with a Promising *In Vivo* Profile Relative to Epothilone B," *Proc Natl Acad Sci USA*, 95:9642-9647 (1998); Chou Ting-Chao, et al., "Desoxyepothilone B is Curative Against Human Tumor Xenografts that are Refractory to Paclitaxel," *Proc Natl Acad Sci USA*, 95:15798-15802 (1998); Hattori H., et al., "Nucleosides and Nucleotides. 158. 1-(3-C-Ethynyl-ß-D-*ribo*-pentofuranosyl)-cytosine,1-(3-3-*C*-Ethynyl-ß-D-*ribo*-pentofuranosyl)uracil, and Their Nucleobase Analogues as New Potential Multifunctional Antitumor Nucleosides with a Broad Spectrum of Activity," *J Med Chem*, 39:5005-5011 (1996); Jesson M.I., et al., "Characterization of the DNA-DNA Cross-Linking Activity of 3'-(3-Cyano-4-morpholinyl)-3'-deaminoadriamycin," *Cancer Res*, 49:7031-7036 (1989); Acton E.M., et al., "Intensely Potent Morpholinyl Anthracyclines," *J Med Chem*, 27:638-645 (1984); Nagy A., et al., "High Yield Conversion of Doxorubicin to 2-pyrrolinodoxorubicin, and Analog 500-1000 Times More Potent: Structure-Activity Relationship of Daunosamine-Modified Derivatives of Doxorubicin," *Proc Natl Acad Sci USA*, 93:2464-2469 (1996); Duch D.S., et al., "Biochemical and Cellular Pharmacology of 1843U89, a Novel Benzoquinazoline Inhibitor of Thymidylate Synthase," *Cancer Res*, 53:810-818 (1993); Panda D., et al., "Antiproliferative Mechanism of Action of Cryptophycin-

52: Kinetic Stabilization of Microtubule Dynamics by High-Affinity Binding to Microtubule Ends," *Proc Natl Acad Sci USA*, 95:9313-9318 (1998); Marsham P.R., et al., "Design and Synthesis of Potent Non-Polyglutamatable Quinazoline Antifolate Thymidylate Synthase Inhibitors," *J Med Chem*, 42:3809-3820 (1999); Nicolaou K.C., et al., "Chemical Biology of Epothilones," *Angew Chem Int Ed*, 37:2014-2045 (1998); Boger D.L.; Cai H., "Bleomycin: Synthetic and Mechanistic Studies," *Angew Chem Int Ed*, 38:448-476 (1999); Leioni L., et al., "Indanocine, a Microtubule-Binding Indanone and a Selective Inducer of Apoptosis in Multi-drug-Resistant Cancer Cells," *J Nat Cancer Inst*, 92(3):217-224 (2000), the contents of which are incorporated herein by reference in their entirety.

Preferred cytotoxins to comprise E are compounds that are cytotoxic to cells at low concentrations, preferably at submicromolar or nanomolar concentrations or subnanomolar concentrations. However, in some preferred embodiments even effector agents that are active at micromolar or higher concentrations may be utilized. This is especially true if the effector agent is operative at a cellular compartment that is targeted by the ET drug. Targeting can result in a profound localized increase in concentration of the effector agent and produce localized concentrations thousands to millions of times higher then the overall concentration.

The scope of the present invention also includes the case where E is comprised of a protein, oligopeptide analog, oligonucleotide analog, polynucleotide analog,

or other molecular species, which would benefit from the targeted delivery methods.

E can also be comprised of a group, with a therapeutic radioisotope or a boron bearing group, for use in neutron capture therapy. Suitable radioactive agents are well known to one skilled in the arts.

E can be connected to the drug complex either by a trigger, which when activated releases it or E can be connected in a stable fashion directly to a linker. The mode of connection depends upon the requirements for E to exert its effector function. For example, if E is a radioisotope liberation form the target drug complex is unnecessary for activity.

Preferrably the connection of the effector agent to the remainder of the drug ET should be by chemical groups that are sufficiently stable in vivo to allow the drug to reach the target site mostly intact. If the effector agent can evoke its intended pharmacological activity while still attached to the remainder of the molecule ET than it is preferable that the connection of E to T be by a chemical linkage that is resistant or significantly resistant to cleavage in vivo. Examples of preferred chemical linkages for this case include: C-C bonds; ether bonds; amides; carbamates; thioethers; C-N bonds; and ureas.

In a preferred embodiment the effector agent E is a cytotoxic drug that is connected to a trigger that is connected to a linker that is connected to the remainder of the drug ET. In a preferred embodiment the trigger is a group that

can be preferentially modified or activated inside cells and releases the cytotoxin inside the cell. Preferred embodiments of triggers are described in the linker section. Other preferred embodiments of triggers are also shown in the Example section. In a preferred embodiment the connection of E to T can be by a chemical linkage that is resistant or significantly resistant to cleavage in vivo but which is cleaved upon in vivo modification or activation of a trigger group. Preferred chemical linkages of an efffector agent to a trigger are by chemical groups such as carbamates, amides, acetals, and ketals, phosphotriesters, phosphonate diesters, and disulfides. Other functionalities such as esters, carbonates, or any other type of chemical linkage that is sufficiently stable in vivo to allow the drug to reach the target site substantially intact may be employed.

Immunological Effector Groups

The present invention can also be used to label target cells for destruction by the immune system. Nature has endowed the body with powerful and effective mechanisms to destroy foreign antigens. The fundamental obstacle to the utilization of these capabilities in the therapy of cancer is the paucity of antigens unique to malignant cells that can trigger an effective immune response. An impressive array of approaches has been utilized to marshal the immune response against tumors with variable results. The following references relate to this subject matter: Vollmer C.M. Jr., et al., "Alpha-Fetoprotein-Specific Genetic Immunotherapy For Hepatocellular Carcinoma," *Cancer Res,* 59(13):3064-7 (1999); Gan Y.H., et al., "Antitumour Immunity of Bacillus Calmette-Guerin and

Interferon Alpha in Murine Bladder Cancer," *Eur J Cancer,* 35(7):1123-9 (1999); Ganss R., et al., "Autoaggression and Tumor Rejection: It takes More than Self-Specific T-Cell Activation," *Immunol Rev,* 169:263-72 (1999); Berd D., et al., "Autologous, Hapten-Modified Vaccine as a Treatment for HumanCancers," *Semin Oncol,* 25(6):646-53 (1998); Greten T.F.; Jaffee E.M., "Cancer Vaccines," *J Clin Oncol,* 17(3):1047-60 (1999); Manzke O., et al., "CD3X Anti-Nitrophenyl Bispecific Diabodies: Universal Immunotherapeutic Tools tor Retargeting T Cells to Tumors." *Int J Cancer,* 82(5):700-8 (1999); Vet J.A., et al., "Comparison of P53 Protein Over-expression with P53 Mutation in Bladder Cancer: Clinical and Biologic Aspects," *Lab Invest,* 73(6):837-43 (1995); Jager E., et al., "CTL-Defined Cancer Vaccines: Perspectives for Active Immunotherapeutic Interventions in Minimal Residual Disease," *Cancer Metastasis Rev,* 18(1):143-50 (1999); Hart D.; Hill G., "Dendritic Cell Immunotherapy for Cancer: Application to Low-Grade Lymphoma and Multiple Myeloma," *Immunol Cell Biol,* 77(5):451-9 (1999); Timmerman J.M.; Levy R., "Dendritic Cell Vaccines for Cancer Immunotherapy," *Annu Rev Med,* 50:507-29 (1999); Tjoa B.A., et al., "Follow-Up Evaluation Of A Phase II Prostate Cancer Vaccine Trial," *Prostate,* 40(2):125-9 (1999); Palmer K., et al., "Gene Therapy with Autologous, Interleukin 2-Secreting Tumor Cells in Patients with Malignant Melanoma," *Hum Gene Ther,* 10(8):1261-8 (1999); Takahashi T., et al.," IgM anti-ganglioside Antibodies Induced by Melanoma Cell Vaccine Correlate with Survival of Melanoma Patients," *J Invest Dermatol,* 112(2):205-9 (1999); Riker A., et al., "Immune Selection after Antigen-Specific Immunotherapy of Melanoma," *Surgery,* 126(2):112-20 (1999); Harris D.T., et al., "Immunologic Approaches to the Treatment of Prostate Cancer," *Semin Oncol,* 26(4):439-47 (1999); Peralta

E.A., et al., "Immunotherapy of Bladder Cancer Targeting P53," *J Urol*, 162(5):1806-11(1999); McGee J.M., et al., "Melanoma Vaccines as a Therapeutic Option," *South Med J*, 92(7):698-704 (1999); Ben-Efraim S., "One Hundred Years of Cancer Immunotherapy: A Critical Appraisal," *Tumour Biol*, 20(1):1-24 (1999); Rickinson A.B., "Targeting Human Tumours with Antigen-Specific Cytotoxic T-Cells," *Br J Cancer*, 80 Suppl 1:51-6 (1999); McCarty T.M., et al., "Targeting P53 for Adoptive T-Cell Immunotherapy," *Cancer Res*, 58(12):2601-5 (1998); Lindauer M., et al., "The Molecular Basis of Cancer Immunotherapy by Cytotoxic T Lymphocytes," *J Mol Med*, 76(1):32-47 (1998); Gilliland L.K., et al., "Universal Bispecific Antibody for Targeting Tumor Cells for Destruction by Cytotoxic T Cells," *Proc Natl Acad Sci U S A*, 85(20):7719-23 (1998), the contents of which are incorporated herein by reference in their entirety.

The immune system is able to destroy tumors via a number of different mechanisms including: cytotoxic CD8+ lymphocytes, CD4+ lymphocytes, NK cells, activated macrophages, neutrophils, antibody dependent cytotoxicity, activated eosinophils, and gamma/ delta T lymphocytes. Antigen specific T cells function as triggers that activate a wide range of antigen nonspecific effectors that can cause profound tissue destruction by antigen nonspecific mechanisms. The importance of nonspecific effector mechanisms in tumor rejection is highlighted by the rejection of MHC II negative melanomas by MHC II restricted CD4+ T cells. The following references relate to this subject matter: Hung K., et al., "The Central Role of CD4+ T Cells in the Antitumor Immune Response," *J Exp Med*, 188(12):2357-2368 (1998); Steinman Lawrence, "A Few Autoreactive

Cells in an Autoimmune Infiltrate Control a Vast Population of Nonspecific Cells: A Tale of Smart Bombs and the Infantry," *Proc Natl Acad Sci USA,* 93:2253-2256 (1996); Greenberg P.D., et al., "Therapy of Disseminated Murine Leukemia with Cyclophosphamide and Immune Lyt-1+,2$^-$ T Cells," *J Exp Med,* 161:1122-1134 (1985); Mumberg, et al., "CD4$^+$ T Cells Eliminate MHC Class II-Negative Cancer Cells *In Vivo* by Indirect Effects of IFN-γ," *Proc Natl Acad Sci USA,* 96:8633-8638 (1999); Qin Z.; Blankenstein T., "CD4$^+$ T Cell-Mediated Tumor Rejection Involves Inhibition of Angiogenesis that is Dependent on IFNγ Receptor Expression by Nonhematopoietic Cells," *Immunity,* 12:677-686 (2000), the contents of which are incorporated herein by reference in their entirety.

The immune system has evolved to allow a small number of antigen specific T cells to orchestrate the destructive activities of a large number of nonspecific effector cells. This has the following profound consequences for the targeted immune destruction of tumors:

1.) The targeted delivery or targeted generation in a tumor of a triggering antigen recognized by sensitized T cells can initiate tumor rejection;

2.) The triggering antigen need not be displayed on tumor cells in a form recognizable by antigen specific T cells;

3.) The triggering antigen can be presented to sensitized antigen specific T cells by macrophages and dendritic cells within the stromal compartment of the tumor and initiate tumor rejection; and

4.) Triggering antigens can be derived from intracellular or extracellular factors in the tumor or tumor microenvironment.

The present invention can allow for the immune system to destroy specifically targeted cells. This can be achieved by delivering to the tumor a variety of immunostimulatory molecules including but not limited to:

1.) Masked antigens;

2.) Masked reactive haptens;

3.) Ligands that result in the formation of neoantigens;

4.) Masked ligands for delta/gamma T cell receptors;

5.) Masked ligands that recruit and mobilize macrophages, monocytes and neutrophils; and

6.) Masked ligands that recruit and activate NK cells.

Masked Antigens

The present invention can allow for an intense immune response to be generated against antigens that are completely unrelated to the tumor and for this immune response to be specifically targeted against the tumor. This can be achieved as follows:

1.) The patient is sensitized to the antigen referred to as "AG" so as to generate high levels of cell mediated immunity against cells bearing the antigen AG;

2.) The antigen AG, masked by one or more bioreversible triggers is selectively delivered to the targeted cell;

3.) After localizing to the target cell via high affinity target cell selective ligands the trigger is activated and the antigen AG is unmasked;

4.) The antigen is then processed either by tumor cells or by macrophages in the tumor microenvironment and complexed to MHC I and or MHC II molecules which trigger the activation of sensitized T cells; and

5.) The antigen activated T cells in the tumor trigger tumor destruction by the recruitment and activation of nonspecific effector cells.

A masked antigen is employed in order to prevent the deactivation of lymphocytes by excess free antigen. The following references relate to this subject matter: Butler L.D., et al., "Unresponsiveness in Hapten-Specific Cytotoxic T Lymphocytes," *J Immunol,* 131(4):1663-1669 (1983), the contents of which are incorporated herein by reference in their entirety.

A pronounced inflammatory reaction can occur at the target site that can amplify the antitumor activity through the innocent bystander effect and other non-selective mechanisms such as vascular thrombosis. This approach can allow target cell destruction without the use of cytotoxic agents. However, the approach can be used in combination with the administration of targeted cytotoxic drugs. The inflammatory reaction, which accompanies the immune response can increase vascular permeability in the tumor microenvironment and facilitate drug penetration into the tumor. The intensity of the immune response can also be amplified by the concurrent administration of a variety of immunomodulators and cytokines such as Interleukins 2, 4, 6, 7, and 15. The following references relate to this subject matter: Vella A.T., et al., "Cytokine-induced Survival of Activated T Cells *In Vitro* and *In Vivo,*" *Proc Natl Acad Sci USA,* 95:3810-3815 (1998); Ayroldi E., et al., "Interleukin-6 (IL-6) Prevents

Activation-Induced Cell Death: IL-2-Independent Inhibition of Fas/fasL Expression and Cell Death," *Blood,* 92(11):4212-4219 (1998), the contents of which are incorporated herein by reference in their entirety.

Targets that are present in the tumor microenvironment but not located on the tumor cell surface can also be employed with this embodiment of the invention. Profound local necrosis due to inflammation and vascular occlusion typified by the Arthus reaction could quite effectively mediate tumor cell death. For example, PSMA is present on the neovasculature of a wide range of malignant tumors including: renal, pancreatic, breast, colon, bladder, testicular carcinoma, melanoma, glioblastoma, and soft tissue sarcomas. Selectively delivering a masked antigen to this site can initiate a delayed hypersensitivity reaction, which would be expected to exert considerable antitumor activity. The following references relate to this subject matter: Chang S.S., et al., "Five Different Anti-Prostate-Specific Membrane Antigen (PSMA) Antibodies Confirm PSMA Expression in Tumor-associated Neovasculature," *Cancer Res,* 59:3192-3198 (1999), the contents of which are incorporated herein by reference in their entirety.

The trigger to unmask the latent antigen AG can be selective or non-selective. The situation is completely analogous to that discussed for masked intracellular transport ligand triggers. The antigen masking trigger serves the following important roles:

1.) It can allow the drug to localize to the target site prior to the initiation of the immune reaction;

2.) It can prevent desensitization of effector lymphocytes by soluble antigen; and

3.) It can allow the intensity of the immune response to be increased by the systemic administration of additional targeted drug bearing masked antigen during the course of an ongoing immune reaction.

Peptide antigens are recognized by lymphocytes in association with major histocompatibility complex (MHC) molecules. Complex antigenic proteins are degraded to peptide fragments that bind to MHC molecules and trigger lymphocyte activation. The binding of antigenic peptides to both MHC class I and MHC class II molecules can occur either intracellularly or extracellulary. Accordingly, the targeted delivery of an antigenic peptide or complex antigen to tumor cells can result in the binding of that antigen or a portion of the antigen by cellular MHC proteins which can mark the cells for immune destruction. T cells with delta gamma receptors recognize antigens directly in the absence of antigen presentation or complexation to MHC molecules. The targeted delivery of an antigen specific for delta gamma T cells could eliminate the need for antigen processing and complexation to tumor cell MHC molecules. The following references relate to this subject matter: Jondal M., et al., "MHC Class I-Restricted CTL Responses to Exogenous Antigens," *Immunity,* 5:295-302 (1996); Schirmbeck R., et al., "Processing of Exogenous Heat-Aggregated (Denatured) and Particulate (Native) Hepatitis B Surface Antigen for Class I-Restricted Epitope Presentation," *J Immunol,* 155:4676-4684 (1995); Schirmbeck R.; Reimann J., "'Empty' L^d Molecules Capture Peptides from Endocytosed Hepatitis B Surface Antigen Particles for Major Histocompatibility

Complex Class I-Restricted Presentation," *Eur J Immunol*, 26:2812-2822 (1996); Santambrogio L., et al., "Extracellular Antigen Processing and Presentation by Immature Dendritic Cells," *PNAS*, 96(26):15056-15061 (1999); Chiu I., et al., "Trafficking of Spontaneously Endocytosed MHC Proteins," *PNAS*, 96(24):13944-13949 (1999); Grommé M., et al., "Recycling MHC Class I Molecules and Endosomal Peptide Loading," *Proc Natl Acad Sci USA*, 96:10326-10331 (1999); Santambrogio L., et al., "Abundant Empty Class II MHC Molecules on the Surface of Immature Dendritic Cells," *PNAS*, 96(26):15050-15055 (1999); Hosken N.A., et al., "Class I-Restricted Presentation Occurs Without Intenalization or Processing of Exogenous Antigenic Peptides," *J Immunol*, 142(4):1079-1083 (1989); Jondal M., et al., "MHC Class I-Restricted CTL Responses to Exogenous Antigens," *Immunity*, 5:295-302 (1996); Yewdell J.W., et al., "Cells Process Exogenous Proteins for Recognition by Cytotoxic T Lymphocytes," *Science*, 239:637-640 (1988); Barlow A.K., et al., "Exogenously Provided Peptides of a Self-antigen Can Be Processed into Forms that Are Recognized by Self-T Cells," *J Exp Med*, 187(9):1403-1415 (1998); Hill A; Ploegh H., "Getting the Inside Out: The Transporter Associated with Antigen Processing (TAP) and the Presentation of Viral Antigen," *Proc Natl Acad Sci*, 92:341-343 (1995); Schirmbeck R., et al., "Similar as well as Distinct MHC Class I-Binding Peptides are Generated by Exogenous and Endogenous Processing of Hepatitis B Virus Surface Antigen," *Eur J Immunol*, 28:4149-4161 (1998); Schirmbeck R., et al., "Injection of Detergent-Denatured Ovalbumin Primes Murine Class I-Restricted Cytotoxic T Cells *in Vivo*," *Eur J Immunol*, 24:2068-2072 (1994); Kovacsovics-Bankowski M., et al., "Efficient Major Histocompatibility Complex Class I Presentation of Exogenous Antigen Upon

Phagocytosis by Macrophages," *Proc Natl Acad Sci USA*, 90:4942-4946 (1993); Song R.; Harding C.V., "Roles of Proteasomes, Transporter for Antigen Presentation (TAP), and β_2-Microglobulin in the Processing of Bacterial or Particulate Antigens Via an Alternate Class I MHC Processing Pathway," *J Immunol*, 156:4182-4190 (1996); Schirmbeck R., et al., "Processing of Exogenous Heat-Aggregated (Denatured) and Particulate (Native) Hepatitis B Surface Antigen for Class I-Restricted Epitope Presentation," *J Immunol*, 155:2676-4686 (1995); Schumacher T.N.M., et al., "Direct Binding of Peptide to Empty MHC Class I Molecules on Intact Cells and In Vitro," *Cell*, 62:563-567 (1990); Staerz U.D., et al., "Cytotoxic T Lymphocytes Against a Soluble Protein," *Nature*, 329:449-451 (1987); Reimann J., et al., "Alternative Processing Pathways for MHC Class I-Restricted Epitope Presentation to $CD8^+$ Cytotoxic T Lymphocytes," *Biol Chem Hoppe-Seyler*, 375:731-736 (1994), the contents of which are incorporated herein by reference in their entirety.

A major advantage of the current approach is the ability to generate an intense immune response against an antigen completely unrelated to the tumor and channel this response against the tumor. The present invention can also be used in conjunction with the in vitro sensitization of the patient's lymphocytes, clonal expansion, and subsequent intravenous infusion of the activated lymphocytes into the patient to adoptively transfer the selected immune response.

The present invention can also be used in conjunction with passively administered antibodies directed against the antigen that is masked. Although

most antibodies are not directly cytotoxic for tumor cells the antibodies can trigger tumor rejection by cell mediated mechanisms. The following references relate to this subject matter: Dyall R., et al., "Cellular Requirements for the Monoclonal Antibody-mediated Eradication of an Established Solid Tumor," *Eur J Immunol,* 29:30-37 (1999); Clynes R., et al., "Fc Receptors are Required in Passive and Active Immunity to Melanoma," *Proc Natl Acad Sci USA,* 95:652-656 (1998), the contents of which are incorporated herein by reference in their entirety.

Key requirement for the selective targeted delivery of an antigen to mark a tumor for destruction by the immune system are as follows:

1.) The antigen can be masked in a bioreversible fashion that allows the drug to localize at the target site prior to antigen unmasking. This can be accomplished functionally in a number of ways:

   a.) The antigen can be chemically masked by a trigger that is activated specifically or nonspecifically in the tumor microenvironment.

   b.) The trigger can be activated by a time clock type trigger, which unmasks the antigen spontaneously at a rate slow enough to allow prior target cell localization.

   c.) The trigger can be unmasked by an enzyme specifically and independently targeted to the tumor.

   d.) Alternatively, the antigen can be generated at the target site by the very interaction of the targeting ligands with target receptors. For example, tamoxifen binding to the estrogen receptor generates new antigenic determinants. The following reference relates to this

subject matter: Martin P.M., et al., "Binding of Antiestrogens Exposes an Occult Antigenic Determinant in the Human Estrogen Receptor," *Proc Natl Acad Sci*, 85:2533-2537 (1988), the contents of which is incorporated herein by reference in its entirety.

2.) The masked chemical moiety can have sufficient molecular size and complexity to function as an antigen following unmasking;

3.) The antigen should preferably be capable of evoking a strong immune response;

4.) The antigen should preferably be a foreign chemical species that does not elicit cross reactivity to normal structures;

5.) The antigen preferably should be a distinct subsite of the molecule which can be seperately used to presensitize the individual without risking sensitization to other portions of the drug; and

6.) The antigen needs to have functionalities, which can be masked and which can prevent antigen recognition until unmasking is triggered.

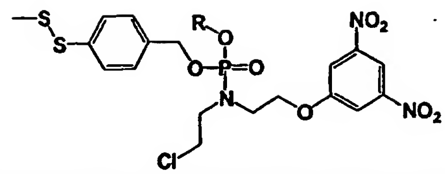
A variety of molecular structures can be employed as a masked antigen. A molecular size comparable to that of an oligopeptide of around 7-8 amino acid groups is required to provide the requisite complexity to elicit a cellular immune response. The following reference relates to this subject matter: Schlossman, S.F; Levine H., "Immunochemical Studies on Delayed and Arthus-Type Hypersensitivity Reactions," *J Immunol*, 98(2):211-219 (1967), the contents of which is incorporated herein by reference in its entirety.

Masked Reactive Haptens

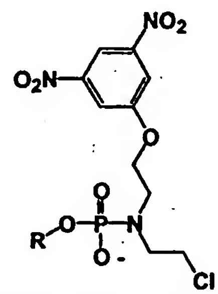
A potential limitation of delivering antigens, which require complexation to MHC proteins for immunogenicity, is the polymorphic nature of the MHC proteins which can impart a significant genetic component to the immune response. This can be addressed by delivering a masked reactive hapten to the tumor that can generate multiple types of haptenized oligopeptides. This can be regarded as analogous to the targeted delivery of a masked contact sensitizing agent. The drug binds to the target cells and a reactive hapten is unmasked which covalently modifies cellular proteins. These hapten modified proteins are then processed and complexed to MHC proteins which trigger the activation of hapten specific sensitized T cells. It is likely that a large number of different hapten modified peptides can be complexed to MHC proteins and recognized by cross reacting hapten sensitized CD4+ and CD8+ T cells. In mice, individual CD4+ T cell clones are able to react to haptens attached to MHC class II molecules via multiple different carrier peptides. The extreme sensitivity and amplification possible by this approach is highlighted by data, which indicates that a single hapten molecule on the surface of a target cell can lead to target cell lysis, by hapten specific lymphocytes. The following references relate to this subject matter: Kohler J., et al., "Cross-reactive Trinitrophenylated Peptides as Antigens for Class II Major Histocompatibility Complex-restricted T Cells and Inducers of Contact Sensitivity in Mice. Limited T Cell Receptor Repertoire," *Eur J Immunol,* 25:92-101 (1995); Sykulev Y.; Joo M.; Vturina I.; Tsomides T.J.; Eisen H.N.; Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response Immunity 6:565-71(1996), the contents of which are incorporated herein by reference in their entirety.

An important feature of this embodiment of the invention is that effector agent E is comprised of one or more groups that are able to covalently modify proteins and components of the tumor. In a preferred embodiment, the chemical reactivity of E is unmasked following the activation of one or more triggers. A large number of chemical entities are able to covalently react with proteins and generate antigenic groups, which can evoke an immune response. Simple haptens such as dinitrophenol can also be coupled to proteins by a large variety of covalent linkers. The mechanisms of covalent modification of cellular proteins compatible with this embodiment of the invention is very broad including the reaction of electrophilies with nucleophilic groups such as thiols, amines, and hydroxy groups of the proteins, the reaction of nucleophiles with electrophilic centers in proteins, and free radical reactions. It is preferrable to employ groups that require triggering to unmask the reactivity required for protein modification. This can allow the drug targeting specificity to be defined by the high affinity interaction of the targeting ligands with the target receptors rather than by the pattern of nonspecific covalent protein modification. The use of chemically stable drugs that require triggering to unmask reactivity also has major practical pharmaceutical advantages. A large number of compounds are known, which require triggering or bioactivation for the unmasking of the chemical reactivity including phosphoramide mustard analogs, quinone methide precursors, enediynes, and nitroimadazoles.

A preferred embodiment (embodiment MRH1) is shown below:

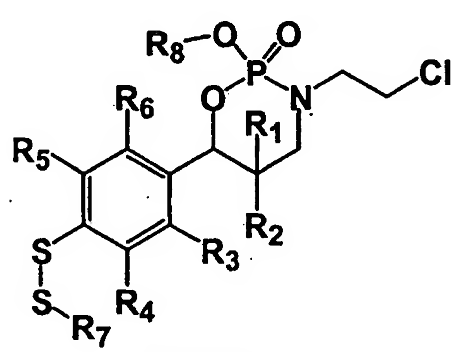


wherein R is the point of linker attachment to the remainder of the target drug complex; cleavage of the disulfide by thiol reductases can release the following compound:



which is an active alkylating agent and can react with nucleophilic groups on adjacent proteins. The conversion of the phosphoester to the negatively charged species enormously increases the nucleophilicity of the adjacent nitrogen and triggers the formation of a highly reactive aziridinium cation, which can rapidly alkylate nucleophiles.

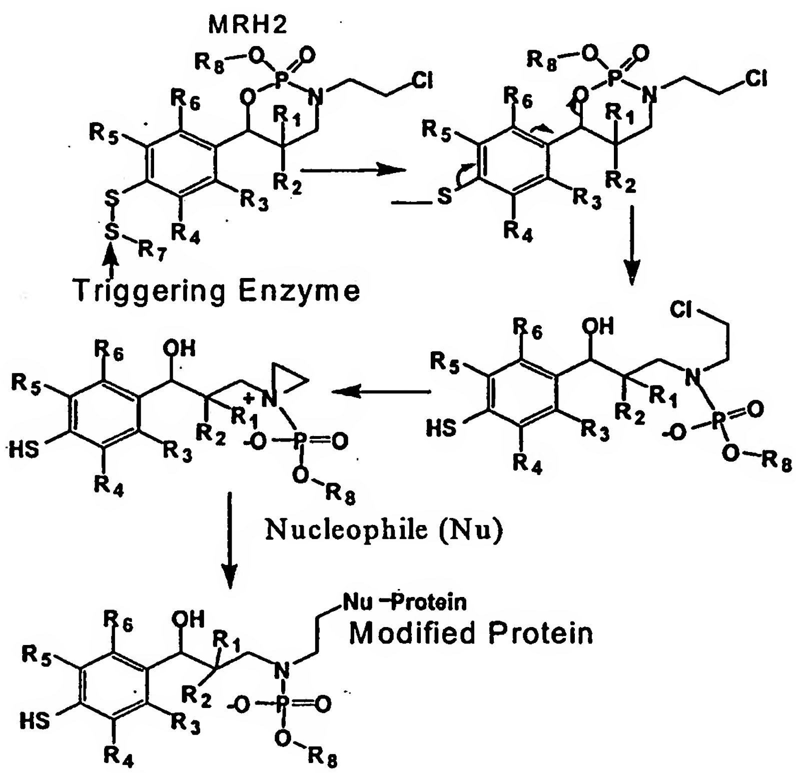
Another preferred embodiment (embodiment MRH2) of E is shown below:



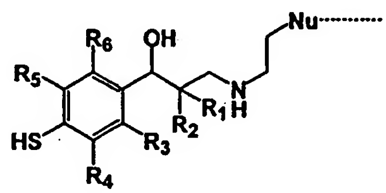
MRH2

wherein R_1 and R_2 can be H, or lower alkyl group; and R_3 to R_6 can be H, Cl, Br, F, I, a nitro group, a lower alkyl group, C_1-C a methoxy or alkoxy group, $-CO_2H$, --CO_2R_9, where R_9 is a lower alkyl group, --$CONHR_9$, -PO_3H_2, -PO_3HR_9, a sulphonic acid group, or other inert groups, which do not interfere with the mechanism of action shown below; and wherein R_7 is an alkyl group, and a phenyl group. R_7-SH can be cysteine or a derivative of cysteine, and R_7 can be a group such that the resulting disulfide is reduced by cells; and wherein R_8 is the point of attachment to the remainder of ET.

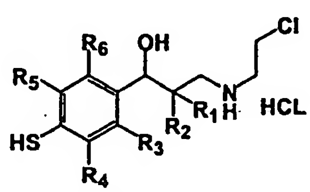
The mechanism of protein modification by this group is shown below wherein Nu represents a nucleophilic group on the protein.



The modified protein can be internalized and degraded by tumor cells and antigen presenting cells such as macrophages in the tumor stroma. The P-N bond is labile and can undergo cleavage. Oligopeptide fragments displaying the hapten shown below can ultimately be presented on the cells in association with MHC I and MHC II molecules.



The patient can be sensitized to this hapten without exposure and without sensitizationto the *masked* hapten. This can be accomplished by immunizing the patient with a compound of the following structure:



This compound can react with cellular proteins and generate the requisite MHC associated hapten derivatized oligopeptide complexes. The patient may also be immunized with biomolecules such as tumor-associated proteins that have been modified by a compound of the above structure, or fragments, or derivatives of such modified molecules. Methods of sensitization are well known to one skilled in the arts and include:

1.) Topical administration;

2.) Intradermal administration with or without adjuvants; or other immunostimulatory agents;

2.) Intradermal administration with or without adjuvants; or other immunostimulatory agents;

3.) Administration of dendritic cells exposed in vitro to the haptenizing agent; and

4.) In vitro sensitization of the patients lymphocytes, clonal expansion in vitro, and infusion of the sensitized cells into the patient.

Targeted Neoantigens Formation

Targeted neoantigen formation is a broadly applicable method, which can allow the immune system to be directed against virtually any factor that is over-expressed by tumor cells or by stromal elements with a tumor. There are an enormous number of proteins and enzymes that are enriched in tumor tissues. However, translating the over-expression of a protein or enzyme into toxicity for the tumor is in general not possible with current technologies. In selected cases, inhibition of an overexpresed enzyme can induce cell death. However, this is by no means the rule. In addition, a large number of proteins are enriched in the tumor microenvironment due to over-expression by stromal elements, rather then tumor cells. Examples include a variety of matrix metalloproteinases. Currently no means exist to convert these microenvironmental factors into selective tumor toxicity. Phase III clinical trials of metalloproteinase inhibitors to date have failed to show antitumor efficacy. The following references relate to this subject matter: Basset P.; Okada A.; Chenard M.P.; Kannan R.; Stoll I.; Anglard P.; Bellocq J.P.; Rio M.C., Matrix metalloproteinases as stromal effectors of human carcinoma progression: therapeutic implications. Matrix

Biol;15:535-41(1997); Genetic Engineering News, No Anti-cancer Benefit in Trials of Marimastat, Feb.15, 2000, the contents of which are incorporated herein by reference in their entirety.

In the most general embodiment targeted neoantigen formation consists of the selective generation of neoantigens by the delivery of a drug that irreversibly chemically modifies the target m. The delivery of a neoantigen forming agent can be by selective or non-selective means. In a preferred embodiment the neoantigen generating effector agent is selectively targeted to the tumor. For certain targets the highly restricted localization of m can allow for tumor-selective neoantigen formation in the absence of other targeting mechanisms. For example, the uniqueness of a prostatic specific antigen to the prostate can allow for a selective mechanism based suicide inhibitor to PSA to be employed for neoantigen generation and targeted immunotherapy. In this case, the delivery of the PSA selective mechanism based suicide inhibitor need not necessarily be by a targeted multifunctional drug delivery vehicle.

In a preferred embodiment, E is comprised of a group that selectively and irreversibly modifies a target selective receptor m(s) and generates a neoantigen(s) (AG) to which an immune response can be generated. The selective interaction between the target receptor m and E confers targeting selectivity to the drug in addition to that provided by the interaction of targeting ligands and target receptors. The molecule m can be a protein, cellular constituent, or biomolecule either inside, on the surface, or in the

microenvironment of tumor cells, which preferably is enriched in the tumor relative to normal tissues.

Table 2 lists some preferred rn for neoantigen formation. It should be noted that the target receptors that bind to the targeting ligands of the drug ET can also be modified and generate neoantigens.

Table 2

Preferred Target Receptors Rn for use in Neoantigen Formation

Enzyme	Malignancies
Prostate specific Antigen	prostate, breast
Human glandular kallikrein 2	prostate, breast
Prostatic acid phosphatase	prostate
Plasmin	numerous
Placental type alkaline phosphatase	ovarian, testicular
Matriptase	breast
Cathepsins	numerous
Matrix metalloproteinases	numerous
Thymidine phosphorylase	numerous
Trypsin	ovarian
Urokinase	numerous
Fatty Acid Synthase	breast , ovarian, prostate, endometrial
Steroid sulfatase	breast, ovarian, endometrial
Epidermal growth factor receptor	numerous

Mitogen activated protein kinase kinase	numerous
Phosphatidylinositol 3-kinase	Breast, lung, prostate, ovarian
Mitogen activated protein kinase	Breast, prostate, colon, ovarian, lung
Mitogen activated protein kinase	Breast , prostate, ovarian ,endometrial
Thymidylate synthase	Colon, cervical, gastric, leukemias, breast
Protein kinase A	Breast, ovarian, lung, colon
Fibroblast activation protein/ seprase	Stroma of breast, colon, lung, ovarian, tumor cells of sarcomas

A general method for converting m into a neoantigen (AG) (or neoantigen precursor) is to employ a drug E-T in which E has the general structure:

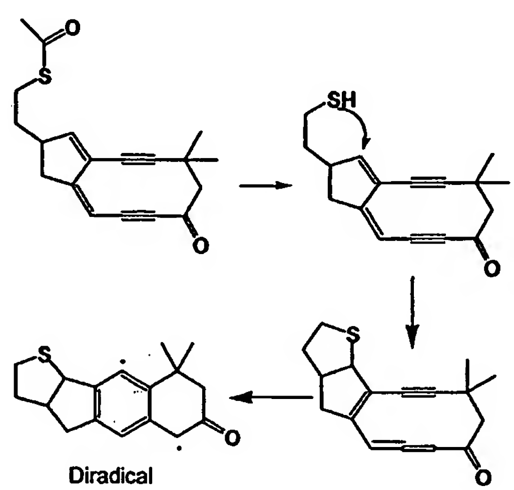
RN-L-V

wherein RN is a group that binds with high affinity to the target m, and L is a linker, and V is a group that can covalently modify the target m; and wherein RN and V are linked together in a manner so as to allow RN to retain binding affinity to m and V to functionally modify m. In a preferred embodiment, V is activated to a reactive form by a clock-like trigger in which the triggering event is followed by the generation of a reactive intermediate over a predictable time course. In another preferred embodiment V is activated by a trigger that is selectively activated by an enzyme that is enriched at the target cell. The reactive intermediate generated upon activation of V can modify m and generate neoantigens either by covalently binding to m or by inducing other covalent

changes in m. For example, V upon activation can generate free radicals that lead to a chemical modification of the target m. The generation of free radicals in the immediate proximity of the target m can result in chemical modification of the target. The extreme reactivity of free radicals can enable modification of sites lacking reactive functionalities. The affinity labeling of proteins with azido and diazo compounds that bind and are then activated with ultraviolet light to generate free radical, is a well known biochemical technique. Neoantigens can also be unmasked by the induction breaks in the peptide chain that lead to peptide fragments that are not generated in the normal course of catabolism of the protein. Ene-diyne anti-cancer drugs damage DNA by the generation of a diradical and are also able to react with proteins. Targeted chelating agents have been reported which modify proteins via free radicals generated by the Fenton reaction. The following references relate to this subject matter: Smith A.L.; Nicolaou K.C. "The enediyne Antibiotics," *J Med Chem,* 39(11):2103-2117 (1996); Wang K.K., "Cascade Radical Cyclizations via Biradicals Generated from Enediynes, Enyne-Allenes, and Enyne-Ketenes," *Chem Rev,* 96:207-222 (1996); Jones G.B., et al., "Understanding Enediyne-Protein Interactions: Diyl Atom Transfer Results in Generation of Aminoacyl Radicals," *Org Lett,* 2(6):811-813 (2000); Hoyer D., et al., "A New Strategy for Selective Protein Cleavage," *J Am Chem Soc,* 112:3249-3250 (1990); Jones G.B., et al., "Target Directed Enediyne Prodrugs: hER and AhR Degradation by a Synthetic Oxo-Enediyne," *Biorg Med Chem Lett,* 6(16):1971-1976 (1996); Schepartz A.; Cuenoud B., et al., "Site-Specific Cleavage of the Protein Calmodulin Using a Trifluoperazine-Based Affinity Reagent," *J Am Chem Soc,* 112:3247-3249 (1990); Hirama M., et al., "Synthesis and DNA-Cleaving Abilities of Functional Neocarzinostatin

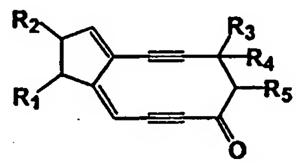
Spontaneous, Low-Temperature Biradical Formation from a Highly Reactive Neocarzinostatin Chromophore-Thiol Conjugate," *J Am Chem Soc,* 1146-1147 (1989); Antoniou A.N., et al., "Control of Antigen Presentation by a Single Protease Cleavage Site," *Immunity,* 12:391-398 (2000); Casciola-Rosen L., et al., "Scleroderma Autoantigens are Uniquely Fragmented by Metal-catalyzed Oxidation Reactions: Implications for Pathogenesis," *J Exp Med,* 185:71-80 (1997); Kalluri R., et al., "Reactive Oxygen Species Expose Cryptic Epitopes Associated with Autoimmune Goodpasture Syndrome," *J Biol Chem,* Mar 23, 2000, the contents of which are incorporated herein by reference in their entirety.

Free radicals can be generated in the course of a variety of cycloaromatization reactions. The following references relate to this subject matter: Hirama M., et al., "Synthesis and Cycloaromatization of a Neocarcinostatin Chromophore Analogue Equipped with an Intramolecular Nucleophile," *Synlett,* 651-653 (1991), the contents of which is incorporated herein by reference in their entirety. For example,



In a preferred embodiment (embodiment V0), V is a triggerable free radical generator.

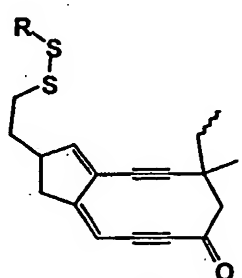
In a preferred embodiment, (embodiment V1) V comprises the following



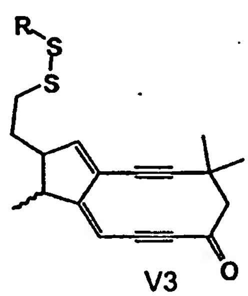
structure:

wherein R_1-R_5 can be H, a lower alkyl group or the site of linker attachment to the remainder of the drug; and wherein R_2 can also be grouped with a masked thiol, amino, carboxylate, or other masked nucleophile that can react with the adjacent double bond and form a 3, 4, 5, or 6 membered ring when unmasked.

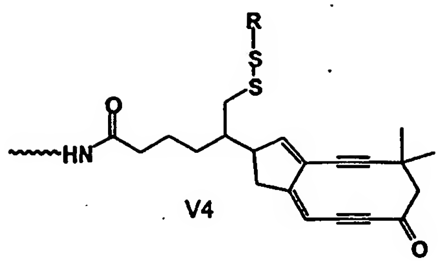
In preferred embodiments (V2, V3, and V4), V comprises the following structures:



V2



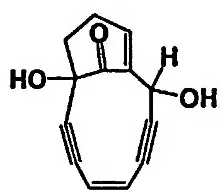
V3



V4

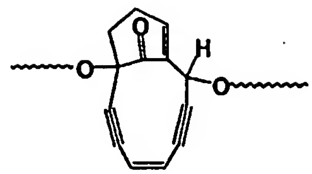
wherein, the dotted line is the site of linker attachement to the remainder of the drug.

A related class of triggerable free radical generators comprises the following structure:



Nucleophilic addition of a thiol triggers a Bergman type cycloaromatization reaction via the intermediacy of a diradical. Compounds of this structure are known to react with proteins. The following references relate to this subject matter: Zein N., et al., "Protein Damage Caused by a Synthetic Enediyne Core," *Biorg Med Chem Lett,* 3(6):1351-1356 (1993); Kadow J.F., et al., "Conjugate Addition-Aldol Approach to the Simple Bicyclic-Diynene Core Structure Found in the Esperamicins and Calicheamicins," *Tetrahedron Lett,* 33(11):1423-1426 (1992), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment (V5), V comprises the following structure:



V5

wherein, the wavy line is a linker or H.

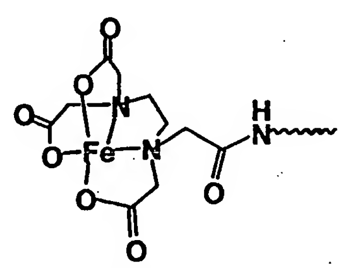
In another preferred embodiment, V is a chelating group that binds a metal capable of catalyzing Fenton like reactions and generating hydroxy radicals or other highly reactive radicals. Human tumor cells can produce large amounts of hydrogen peroxide that can generate hydroxy radicals via the Fenton reaction. In addition, under aerobic conditions, the autooxidation of metal complexes can generate hydroxy free radicals. In the presence of the reducing agent ascorbic acid, a redox cycle can be established leading to augmented hydroxy radical production. Ascorbic acid is generated intracellularly by the reduction of dehydroascorbic acid. Dehydroascorbic acid is transported into cells by the Glut 1 and Glut 3 transporter proteins, which are over-expressed in a wide range of malignancies. Accordingly, the combination of a free radical generator, based on a chelating agent ion complex, used in combination with the administration of ascorbic acid or dehydroascorbic acid, can have synergystic activity and enhanced selectivity for Glut 1 and Glut 3 positive tumors. The following references relate to this subject matter: Szatrowski T.P.; Nathan C.F., "Production of large Amounts of Hydrogen Peroxide by Human Tumor Cells," *Cancer Res,* 51(3):794-8 (1991); Samuni, A., et al., "On the Cytotoxicity of Vitamin C and Metal Ions," *Eur J Biochem,* 137:119-124 (1983); Klebanoff S.J.,

et al., "Oxygen-based Free Radical Generation by Ferrous Ions and Deferoxamine," *J Biological Chem,* 264(33):19765-19771 (1989); Ito, T., et al., "Expression of Facilitative Glucose Transporter Isoforms in Lung Carcinomas: its Relation to Histologic Type, Differentiation Grade, and Tumor Stage," *Mod Pathol,* 11(5):437-43 (1998); Baer S.C., et al., "Expression of the Human Erythrocyte Glucose Transporter Glut1 in Cutaneous Neoplasia," *J Am Acad Dermatol,* 37(4):575-7 (1997); Younes M., et al., "Over-expression of Glut1 and Glut3 in Stage I Nonsmall Cell Lung Carcinoma is Associated with Poor Survival," *Cancer,* 80(6):1046-51 (1997); Younes M., et al., "GLUT1 Expression in Human Breast Carcinoma: Correlation with Known Prognostic Markers," *Anti-cancer Res,* 15(6B):2895-8 (1995); Haber R.S., et al., "GLUT1 Glucose Transporter Expression in Colorectal Carcinoma: A Marker for Poor Prognosis," *Cancer,* 83(1):34-40 (1998); Burstein D.E., et al., "GLUT1 Glucose Transporter: A Highly Sensitive Marker of Malignancy in Body Cavity Effusions," *Mod Pathol,* 11(4):392-6 (1998); Younes M., et al., "Immunohistochemical Detection of Glut3 in Human Tumors and Normal Tissues," *Anti-cancer Res,* 17(4A):2747-50 (1997); Grover-McKay M., et al., "Role for Glucose Transporter 1 Protein in Human Breast Cancer," *Pathol Oncol Res,* 4(2):115-20 (1998); Younes M., et al., "Wide Expression of the Human Erythrocyte Glucose Transporter Glut1 in Human Cancers," *Cancer Res,* 56(5):1164-7 (1996), the contents of which are incorporated herein by reference in their entirety.

Iron (II) complexes with chelating agents are known to generate free radicals under a variety of conditions. The following references relate to this subject matter: Kocha T., et al., "Hydrogen Peroxide-mediated Degradation of protein:

Different Oxidation Modes of Copper- and Iron-dependent Hydroxyl Radicals on the Degradation of Albumin," *Biochem Biophys Acta,* 1337:319-326 (1997); Egan T.J., et al., "Catalysis of the Haber-Weiss Reaction by Iron-Diethylenetriaminepentaacetate," *J Inorg Biochem,* 48:241-249 (1992); Hertzberg R.P.; Dervan P.B., "Cleavage of DNA with Methidiumpropyl-EDTA-Iron(II): Reaction Conditions and Product Analyses," *Biochemistry,* 23:3934-3945 (1984); Schepartz A.; Cuenoud B., "Site-Specific Cleavage of the Protein Calmodulin Using a Trifluoperazine-Based Affinity Reagent," *J Am Chem Soc,* 112:3247-3249 (1990), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment (V6), V is iron complexed with a chelating agent. In a preferred embodiment (V7), V comprises the following structure:



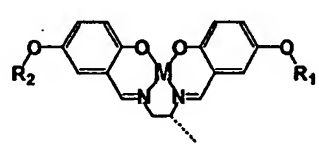
V7

wherein the wavy line is the site of linker attachment to RN.

Salen copper and salen iron complexes are known to generate free radicals under a variety of conditions. The presence of ortho or para hydroxy substituents on the salicylidene moieties leads to a radical generating system from oxygen. The hydroxy substituted salicylidene moieties form hydroquinones,

that cooperate in the redox reaction and aid in the generation of free radicals. Intracellularly a variety of mechanisms exist that can lead to redox cycling and the continued generation of free radicals. The following references relate to this subject matter: Lamour E., et al., "Oxidation of Cu^{II} to Cu^{III}, Free Radical Production, and DNA Cleavage by Hydroxy-Salen-Copper Complexes. Isomeric Effects Studied by ESR and Electrochemisty," *J Am Chem Soc,* 121:1862-1869 (1999); Routier S., et al., "DNA Cleavage by Hydroxy-Salicylidene-Ethylendiamine-Iron Complexes," *Nucleic Acids Res,* 27(21):4160-4166 (1999); Routier S., et al., "Synthesis of a Functionalized Salen-Copper Complex and Its Interaction with DNA," *J Org Chem,* 61:2326-2331 (1996); Routier S., et al., "Synthesis, DNA Binding, and Cleaving Properties of an Ellipticine-Salen Copper Conjugate," *Bioconjugate Chem,* 8:789-792 (1997), the contents of which are incorporated herein by reference in their entirety.

In another preferred embodiment (V8), V comprises the following structure:

V8 

Wherein M is iron (II) or copper (II) and the dotted line is the site of linker attachment to RN; and wherein R_1 and R_2 are H or R_1 and R_2 are bioreversable masking groups for the p-hydroxy groups. In a preferred embodiment, R_1 and R_2 are acyl groups. Cleavage of the esters unmasks p-hydroxy groups, which can trigger the reaction of the complex with oxygen and targeted free radical formation.

Another general method to convert m into a neoantigen is to use a drug E-T in which E is comprised of a mechanism based suicide inhibitor of the target m. Mechanism based suicide inhibitors are a class of enzyme inhibitors that are converted by the catalytic activity of an enzyme into a product that irreversibly modifies and inactivates the enzyme.

Patients can be sensitized to the neoantigen either by immunization with the covalently modified target protein or with synthetic oligopeptides that correspond to the modified portion of the targeted proteins. The target protein m can be modified in a defined manner and can generate defined and identifiable modified oligopeptide fragments upon intracellular proteolytic processing. The patient can also be immunized with these modified oligopeptide fragments. The advantage of this approach is that small chemically defined oligopeptides that correspond to the actual neoantigens, presented by host MHC molecules, can be employed for sensitization rather then complex proteins. The neoantigens generated can be characterized by employing standard labeling and biochemical techniques commonly used to identify the site of affinity labeling of enzymes.

Prostate Specific Antigen Targeted Neoantigens

Prostatic adenocarcinoma cells produce prostatic specific antigen (PSA), a serine protease, which is released into the tumor microenvironment. PSA is both a clinically useful marker for prostate cancer and an attractive target for prostate cancer therapies since the enzyme is expressed in large quantities by a high

percentage of prostate cancers. Doxorubicin prodrugs, designed to be selectively activated by PSA, have been described. PSA is rapidly inactivated by alpha 2-macroglobulin and alpha 1-antichymotrypsin in the circulation. A variety of vaccination approaches against PSA have been developed and are in clinical trials.

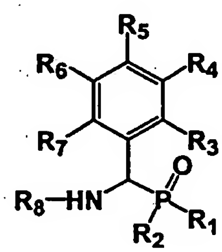
PSA is a chymotrypsin-like serine protease with a preference for the cleavage of the tyrosine —serine bond in oligopeptides of the sequence Ser-Ser-Phe-Tyr—Ser. Other peptide sequences such as His-Ser-Ser-Lys-Leu-Gln—X are also substrates. The catalytic site of PSA bears striking similarity to chymotrypsin, human glandular kallikrein, and tonin. Serine proteases, as a family, are characterized by conserved features in the catalytic active site and are subject to irreversible inactivation by a variety of well-studied mechanism based suicide inhibitors. The very action of the enzyme on the inhibitor results in covalent modification of the enzyme and generates a neoantigen or neoantigen precursor. A neoantigen precursor yields a neoantigen upon cellular proteolytic processing. The following references relate to this subject matter: Coombs G.S., et al., "Substrate Specificity of Prostate-Specific Antigen (PSA)," *Chem & Biol,* 5(9):475-488 (1998); Villoutreix B.O., et al., "A Structural Model for the Prostate Disease Marker, Human Prostate-specific Antigen," *Protein Sci,* 3:2033-2044 (1994); Vihinen, Mauno, "Modeling of Prostate Specific Antigen and human Glandular Kallikrein Structures," *Biochem Biophys Res Comm,* 204(3):1251-1256 (1994); Denmeade S.R., et al., "Enzymatic Activation of a Doxorubicin-Peptide Prodrug by Prostate-Specific Antigen," *Cancer Res,* 58:2537-2540 (1998); Christensson A., et al., "Enzymatic Activity of Prostate-Specific Antigen

and its Reactions with Extracellular Serine Proteinase Inhibitors," *Eur J Biochem,* 194(3):755-63 (1990); Zhang W.M., et al., "Characterization and Immunological Determination of the Complex Between Prostate-Specific Antigen and Alpha2-Macroglobulin," *Clin Chem,* 44(12):2471-9 (1998); Meidenbauer N., et al., "Generation of PSA-reactive Effector Cells after Vaccination with a PSA-based Vaccine in Patients with Prostate Cancer," *Prostate,* 43(2):88-100 (2000); Correale P., et al., "In Vitro Generation of Human Cytotoxic T Lymphocytes Specific for Peptides Derived from Prostate-Specific Antigen," *J Natl Cancer Inst,* 89(4):293-300 (1997); Sanda M.G., et al., "Recombinant Vaccinia-PSA (PROSTVAC) can Induce a Prostate-Specific Immune Response in Androgen-Modulated Human Prostate Cancer," *Urology,* 53(2):260-6 (1999); Slovin S.F.; Scher H.I., "Peptide and Carbohydrate Vaccines in Relapsed Prostate Cancer: Immunogenicity of Synthetic Vaccines in Man--Clinical Trials at Memorial Sloan-Kettering Cancer Center," *Semin Oncol,* 26(4):448-54 (1999), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment (Eneo1), the effector group E of the drug E-T comprises a mechanism-based inhibitor of PSA. Alpha-(aminoalkyl)phosphonate diphenyl esters irreversibly inactivates serine proteases by phosphonylating serine in the catalytic site. The following references relate to this subject matter: Oleksyszyn, Jozef; Powers, James C., "Irreversible Inhibition of Serine Proteases by Peptidyl Derivatives of α–Aminoalkylphosphonate Diphenyl Esters," *Biochem Biophys Res Comm,* 161(1):143-149 (1989); Oleksyszyn, Jozef; Powers, James C., "Irreversible Inhibition of Serine Proteases by Peptide Derivatives of (α-Aminoalkyl)phosphonate Diphenyl Esters," *Biochem,* 30:485-

493 (1991); Oleksyszyn J., et al., "Novel Amidine-Containing Peptidyl Phosphonates as Irreversible Inhibitors for Blood Coagulation and Related Serine Proteases," *J Med Chem,* 37:226-231 (1994), the contents of which are incorporated herein by reference in their entirety.

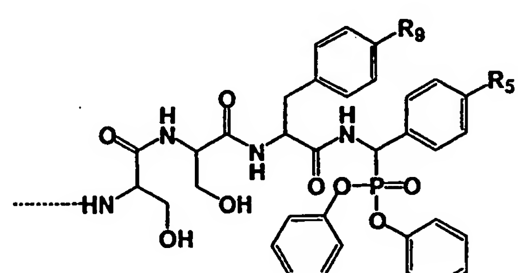
In a preferred embodiment (Eneo2), E is comprised of a compound given by the following structure:



wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus; and wherein R_3-R_7 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_8 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

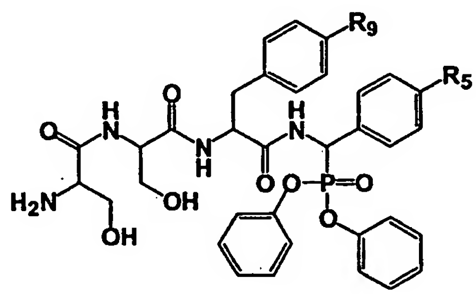
In a preferred embodiment (Eneo3), E comprises the following structure:

Eneo3



wherein R_5 and R_9 are H, or OH, and wherein the dashed line indicates the site of linker attachment to the remainder of the drug.

The inhibition of PSA, by a compound of the above structure, can give rise to a family of neoantigens derived from PSA in which the hydroxy group of the serine of the catalytic triad is phosphonylated. Patients can be sensitized to these neoantigens by immunization with PSA that has been modified by treatment with an inhibitor of related structure such as:

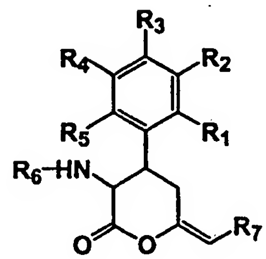


Alternatively, the pateint can be sensitized by immunization with oligopeptide fragments containing the region of the PSA protein that bears the phosphonylated serine residue. The primary amino acid sequence of PSA is known; therefore, the sequence of the neoantigen family is also known.

In another preferred embodiment E is a haloenol based mechansim based suicide inhibitor of PSA. Haolenol lactones are a class of irreversible serine protease inhibitors, which alkylate the enzyme. The following references relate to this subject matter: Baek D.J., et al., "Alternate Substrate Inhibitors of an alpha-Chymotrypsin: Enantioselective Interaction of Aryl-Substituted Enol Lactones," *Biochemistry,* 29(18): 4305-11 (1990); Sofia M.J., et al., "Enol

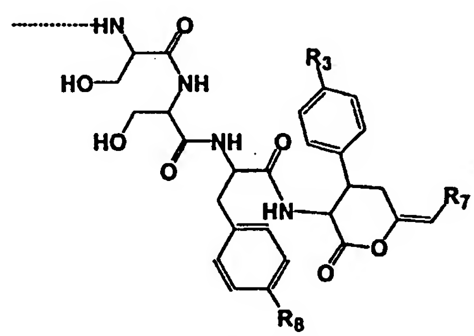
Lactone Inhibitors of Serine Proteases. The Effect of Regiochemistry on the Inactivation Behavior of Phenyl-Substituted (Halomethylene)Tetra- and -Dihydrofuranones and (Halomethylene) Tetrahydropyranones Toward Alpha-Chymotrypsin: Stable Acyl Enzyme Intermediate," *J Med Chem,* 29(2):230-8 (1986); Raj R., et al., "Guanidinophenyl-Substituted Enol Lactones as Selective, Mechanism-Based Inhibitors of Trypsin-Like Serine Proteases," *J Med Chem,* 35(22):4150-9 (1992); Baek D.J. and Katzenellenbogen J.A., "Halo Enol Lactone Inhibitors of Chymotrypsin: Burst Kinetics and Enantioselectivity of Inactivation," *Biochem Biophys Res Commun,* 178(3):1335-42 (1991); Reed P.E., et al., "Proline-Valine Pseudo Peptide Enol Lactones. Effective and Selective Inhibitors of Chymotrypsin and Human Leukocyte Elastase," *J Biol Chem,* 266(1):13-21 (1991); Daniels S.B. et al., "Halo Enol Lactones: Studies on the Mechanism of Inactivation of Alpha- Chymotrypsin," *Biochem,* 25(6):1436-44 (1986); Rai R. and Katzenellenbogen J.A., "Effect of Conformational Mobility and Hydrogen-Bonding Interactions on the Selectivity of Some Guanidinoaryl-Substituted Mechanism-Based Inhibitors of Trypsin-like Serine Proteases," *J Med Chem,* 35:4297-4305 (1992); Daniels S.B., et al., "Haloenol Lactones," *J Biol Chem,* 258(24):15046-15053 (1983); Baek DJ, et al., "Alternate Substrate Inhibitors of Chymotrypsin: Enantioselective Interaction of Aryl-Substituted Enol Lactones," *Biochem,* 29:4305-4311 (1990); Baek DJ and Katzenellenbogen J.A., "Halo Enol Lactone Inhibitors of Chymotrypsin: bust Kinetics and Enantioselectivity of Inactivation," *Biochem Biophys Res Comm,* 178(3):1335-1342 (1991), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment (Eneo4), E is comprised of a compound given by the following structure:



wherein R_1-R_5 can be H, Cl, Br, F, I , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_6 is an oligopeptide or oligopeptide analog connected to the remainder of the drug, or wherein R_6 is a linker connected to the remainder of the drug, and wherein R_7 is Cl, Br, F, I, .

In a preferred embodiment (Eneo5), R_1, R_2, R_4, and R_5 are H, and R_3 is H or OH. In another preferred embodiment, E comprises the following structure:



wherein R_3 and R_8 are H, or OH, and the dashed line indicates the site of linker attachment to the remainder of the drug.

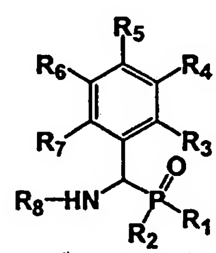
Human Glandular Kallikrein 2 Targeted Neoantigens

Human glandular kallikrein 2 (HGK2) is a prostate specific serine protease that is closely related to PSA. HGK2 cleaves amide bonds adjacent to arginine residues. For example, H-D-Pro-Phe-Arg-p-nitroanilide is cleaved by HGK2. HGK2 is an excellent marker for prostate cancer that is over-expressed in essentially all prostate cancer. Increased invasiveness of prostate cancer is accompanied by increased expression of HGK2. HGK2, like PSA, is also expressed in a significant proportion of human breast cancers. The enzyme is rapidly inactivated by normal plasma protease inhibitors. Currently no methodology exists to exploit the tremendous potential of HGK2 as a target for the therapy of prostate and breast cancer. The following references relate to this subject matter: Heeb M.J., et al., "alpha2-Macroglobulin and C1-Inactivator are Plasma Inhibitors of Human Glandular Kallikrein," *Blood Cells Mol Dis,* 24(4):412-419 (1998), Grauer L.S., et al., "Detection of Human Glandular Kallikrein, Hk2, as its Precursor Form and in Complex with Protease Inhibitors in Prostate Carcinoma Serum," *J Androl,* 19(4):407-11 (1998), Kumar A., et al., "Expression of Human Glandular Kallikrein, hK2, in Mammalian Cells," *Cancer Res,* 56(23):5397-402 (1996); Darson M.F., et al., "Human Glandular Kallikrein 2 (hk2) Expression in Prostatic Intraepithelial Neoplasia and Adenocarcinoma: A Novel Prostate Cancer Marker," *Urology,* 49(6):857-62 (1997); Darson M.F., et al., "Human Glandular Kallikrein 2 Expression in Prostate Adenocarcinoma and Lymph Node Metastases," *Urology,* 53(5):939-44 (1999); Mikolajczyk S.D., et al., "Human Glandular Kallikrein, hk2, Shows Arginine-Restricted Specificity and Forms Complexes with Plasma Protease Inhibitors," *Prostate,* 34(1):44-50 (1998); Grauer L.S., et al., "Identification of Human Glandular Kallikrein hHk2

from LNCaP Cells," *J Androl,* 17(4):353-9 (1996); McGarvey T., et al., "In Situ Hybridization Studies of Alpha 2-Macroglobulin Receptor and Receptor-Associated Protein in Human Prostate Carcinoma," *Prostate,* 28(5):311-7 (1996); Saedi M.S., et al., "Over-expression of a Human Prostate-Specific Glandular Kallikrein, hk2, In E. Coli and Generation of Antibodies," *Mol Cell Endocrinol,* 109(2):237-41 (1995), the contents of which are incorporated herein by reference in their entirety.

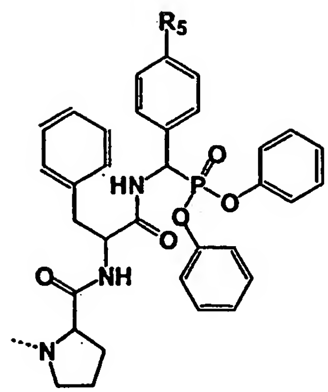
In a preferred embodiment of the invention, E is a mechanism based suicide inhibitor for HGK2.

In a preferred embodiment (Eneo6), E comprises the following structure:



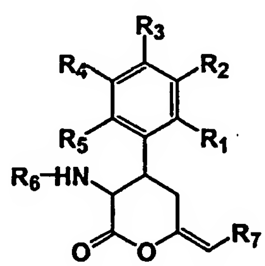
wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus; and wherein R_3, R_4, R_6, and R_7 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_5 is an amidino group, a guanidino group, or a positively charged group, and R_8 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment (Eneo7), E comprises the following structure:



wherein R_5 is an amidino group, or a guanidino group.

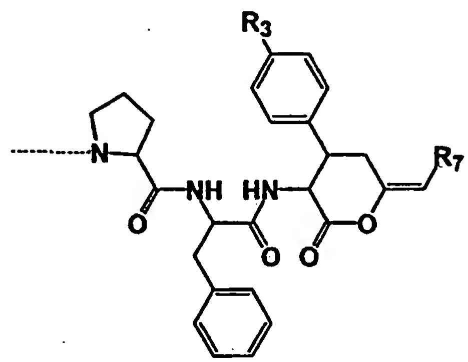
In another preferred embodiment, E is a haloenol mechansim based suicide inhibitor of HGK2. Haolenol lactones are a class of irreversible serine protease inhibitors that alkylate the enzyme. In a preferred embodiment (Eneo8), E is comprised of a compound given by the following structure:



wherein R_1, R_2, R_4, and R_5 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_3 is an amidino group, a guanidino group, or a positively charged group. R_7 is Cl, Br, F, I, and R_6 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment designated as (Eneo9), R_1, R_2, R_4, and R_5 are H, and R_3 is an amidino, or guanidino group.

In a preferred embodiment (Eneo10), E comprises the following structure:



wherein R_3 is an amidino or a guanidino group and R_7 is Cl, Br, F, I, and the dotted line is the site of linker attachment to the remainder of the drug.

Patients can be sensitized to the neoantigens that arise from the modification of HGK2 by inhibitors using the same approaches as described for PSA derived neoantigens.

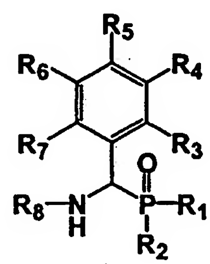
Plasmin Targeted Neoantigens

Most human malignancies are characterized by the elevated expression of urokinase and tissue plasminogen activator that results in the activation of plasminogen into plasmin. Tumor-associated plasmin can serve as an excellent tumor marker for neoantigen directed therapy. Plasmin is a serine protease with specificity for cleaving amide bonds adjacent to lysine and arginine. For

example, benzyloxycarbonyl-D-Ile-Phe-Lys—p-nitroanilide is an excellent substrate for plasmin.

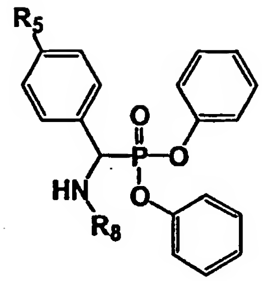
In a preferred embodiment of the invention, E is a mechanism based suicide inhibitor for plasmin.

In a preferred embodiment (Eneo11), E comprises the following structure:



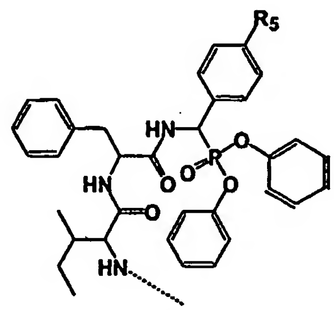
wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus; and wherein R_3, R_4, R_6, and R_7 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_5 is an amidino group, a guanidino group, or a positively charged group, and R_8 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment (Eneo12), E comprises the following structure:



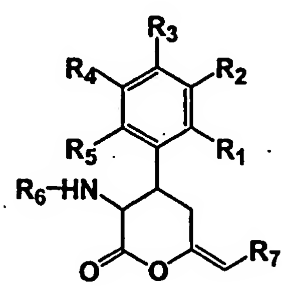
wherein R_5 is an amidino or guanidino group.

In another preferred embodiment (Eneo13), E comprises the following structure:



wherein R_5 is an amidino or guanidino group, and the dotted line is the site of linker attachment to the remainder of the drug.

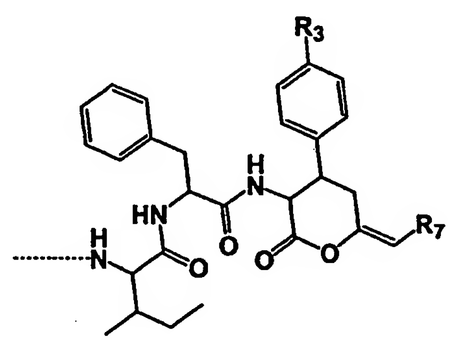
In another preferred embodiment, E is a haloenol mechansim based suicide inhibitor of plasmin. In a preferred embodiment (Eneo14), E is comprised of a compound given by the following structure:



wherein R_1, R_2, R_4, and R_5 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_3 is an amidino group, a guanidino group, or a positively charged group. R_7 is Cl, Br, F, I, and R_6 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment (Eneo15), R_1, R_2, R_4, and R_5 are H, and R_3 is an amidino, or guanidino group.

In another preferred embodiment (Eneo16), E comprises the following structure:



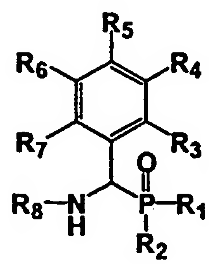
wherein R_3 is an amidino or a guanidino group and R_7 is Cl, Br, F, I, and the dotted line is the site of linker attachment to the remainder of the drug.

Urokinase Targeted Neoantigens

Urokinase is a serine protease, which is over-expressed by most human malignancies and functions to activate plaminogen into plasmin on the surface of tumor cells. Urokinase preferentially cleaves amide bonds adjacent to arginine and lysine residues.

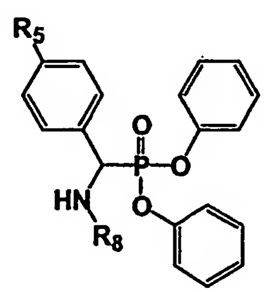
In a preferred embodiment (Eneo17), E is a mechanism based suicide inhibitor for urokinase.

In a preferred embodiment (Eneo18), E comprises the following structure:



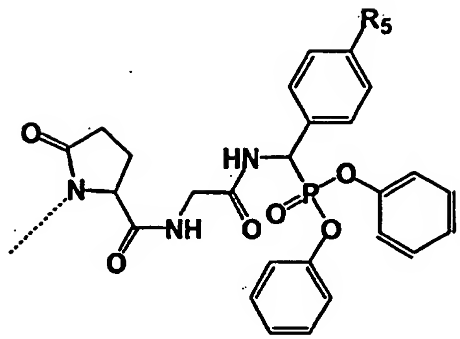
wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus; and wherein R_3, R_4, R_6, and R_7 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_5 is an amidino group, a guanidino group, or a positively charged group and R_8 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment (Eneo18), E comprises the following structure:



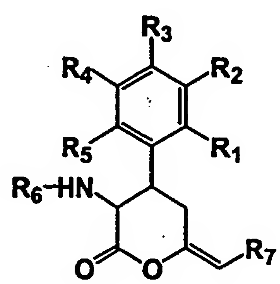
wherein R_5 is an amidino or guanidino group.

In another preferred embodiment (Eneo19), E comprises the following structure:



wherein R_5 is an amidino or guanidino group, and the dotted line is the site of linker attachment to the remainder of the drug.

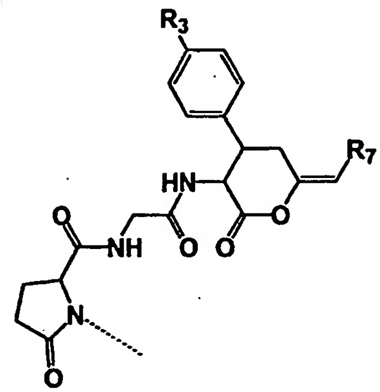
In another preferred embodiment, E is a haloenol mechansim based suicide inhibitor of urokinase. In a preferred embodiment (Eneo20) E is comprised of a compound given by the following structure:



wherein R_1, R_2, R_4, and R_5 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_3 is an amidino group, a guanidino group, or a positively charged group. R_7 is Cl, Br, F, I, and R_6 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment, R_1, R_2, R_4, and R_5 are H, and R_3 is an amidino, or guanidino group.

In another preferred embodiment (Eneo21), E comprises the following structure:



Wherein R_3 is an amidino or a guanidino group and R_7 is Cl, Br, F, I, and the dotted line is the site of linker attachment to the remainder of the drug.

Matriptase Targeted Neoantigens

Matriptase is a typical serine protease with gelatinase activity that is expressed on the surface of breast cancer cells. The enzyme like trypsin and urokinase cleaves preferentially amide bonds adjacent to arginine or lysine residues. The following references relate to this subject matter: Lin C.Y., et al.," Characterization of a Novel, Membrane-Bound, 80-kDa Matrix-Degrading Protease from Human Breast Cancer Cells. Monoclonal Antibody Production, Isolation, and Localization," *J Biol Chem,* 272(14):9147-52 (1997); Lin C.Y., et al., "Molecular Cloning of cDNA for Matriptase, a Matrix-Degrading Serine Protease with Trypsin-Like Activity," *J Biol Chem,* 274(26):18231-6 (1999); Lin C.Y., et al., "Purification and Characterization of a Complex Containing Matriptase and a Kunitz-Type Serine Protease Inhibitor from Human Milk," *J Biol*

Matriptase and a Kunitz-Type Serine Protease Inhibitor from Human Milk," *J Biol Chem*, 274(26):18237-42 (1999), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a mechanism based suicide inhibitor for matriptase. The same structures described above for urokinase can be employed to generate neoantigens for matriptase.

Fibroblast Activation Protein Targeted Neoantigens

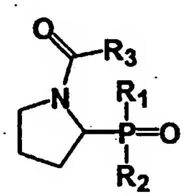
Fibroblast Activation Protein (FAP) is a serine protease with gelatinase and prolyl oligopeptidase activity. FAP is expressed on the surface of tumor-associated fibroblasts in the vast majority of human malignancies including: breast, colon, lung, ovarian, and pancreatic cancer. In addition, the enzyme is present on the surface of human malignancies of mesenchymal origin such as fibrosarcomas and osteogenic sarcomas. FAP is also expressed on fibroblasts during wound healing. The potential of FAP as an almost universal tumor target has been appreciated for many years but remains to be exploited. These considerations make FAP an excellent tumor-associated target for neoantigen directed immunotherapy. As discussed previously, the induction of an intense immune reaction in the tumor stroma can exert pronounced antitumor activity by nonspecific mechanisms. For sarcomas and other FAP+ tumor cell types, the drug can be targeted to the tumor cells. For tumors in which the stromal cells are FAP positive and the tumor cells are FAP negative, the drug can be targeted to other features of tumor-associated fibroblasts by the targeting ligands of ET.

Numerous other targets are known to be enriched on tumor-associated fibroblasts including a variety of matrix metalloproteinases and collagenases. The following references relate to this subject matter: Niedermeyer J., et al., "Mouse Fibroblast-Activation Protein – Conserved Fap Gene Organization and Biochemical Function as a Serine Protease," *Eur J Biochem,* 254(3):650-4 (1998); Park J.E., et al., "Fibroblast Activation Protein, a Dual Specificity Serine Protease Expressed in Reactive Human Tumor Stromal Fibroblasts," *J Biol Chem,* 274(51):36505-36512 (1999); Mueller S.C., et al., "A Novel Protease-docking Function of Integrin at Invadopodia," *J Biol Chem,* 274(35):24947-24952 (1999); Scanlan M.J., et al., "Molecular Cloning of Fibroblast Activation Protein α, a Member of the Serine Protease Family Selectively Expressed in Stromal Fibroblasts of Epithelial Cancers," *Proc Natl Acad Sci USA,* 91:5657-5661 (1994); Goldstein L.A., et al., "Molecular Cloning of Seprase: a Serine Integral Membrane Protease from Human Melanoma, *Biochim Biophys Acta,* 1361(1):11-9 (1997); Rettig W.J., et al., "Fibroblast Activation Protein: Purification, Epitope Mapping and Induction by Growth Factors," *Int J Cancer,* 58(3):385-92 (1994); Levy M.T., et al., "Fibroblast Activation Protein: a Cell Surface Dipeptidyl Peptidase and Gelatinase Expressed by Stellate Cells at the Tissue Remodelling Interface in Human Cirrhosis," *Heptagoloty,* 29(6):1768-78 (1999); Rettig W.J., et al., "Regulation and Heteromeric Structure of the Fibroblast Activation Protein in Normal and Transformed Cells of Mesenchymal and Neuroectodermal Origin," *Cancer Res,* 50(14):3327-35 (1993); Niedermeyer J., et al., "Targeted Disruption of Mouse Fibroblast Activation Protein," *Molec Cell Biol,* 20(3):1089-1094 (2000); Welt S., et al., "Antibody Targeting in Metastatic Colon Cancer: a Phase I Study of Monoclonal Antibody F19 Against

12(6):1193-203 (1994); Garin-Chesa P., et al., "Cell Surface Glycoprotein of Reactive Stromal Fibroblasts as a Potential Antibody Target in Human Epithelial Cancers," *Immunology,* 87:7235-7239 (1990), the contents of which are incorporated herein by reference in their entirety.

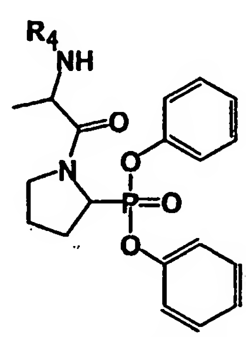
In a preferred embodiment, E is a mechanism based suicide inhibitor for FAP.

In a preferred embodiment (Eneo22), E comprises the following structure:



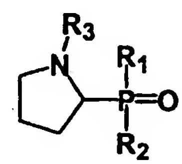
wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus. R_3 is an oligopeptide, oligopeptide analog, or a linker connected to the remainder of the drug.

In a preferred embodiment (Eneo23), E comprises the following structure:



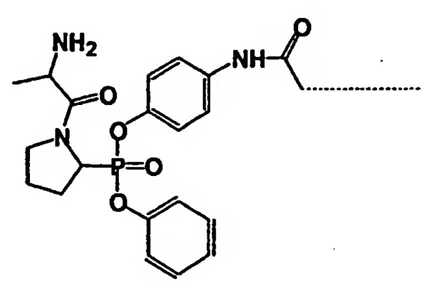
wherein R_4 is the site of linker attachment to the remainder of ET.

In another preferred embodiment (Eneo25), E comprises the following structure:



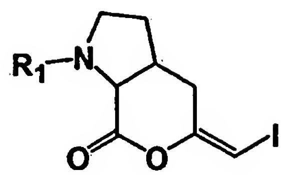
wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus. R_3 is an amino acid, coupled via its carboxylic group, and wherein either R_1 or R_2 has a site to which a linker is attached to the remainder of the drug.

In a preferred embodiment (Eneo26), E comprises the following structure:



Wherein the dotted line is the site of linker attachment to the remainder of the drug.

In another preferred embodiment, E is a haloenol mechansim based suicide inhibitor of FAP. In a preferred embodiment (Eneo27), E is comprised of a compound given by the following structure:



wherein R_1 is an oligopeptide, oligopeptide analog, or a linker connected to the remainder of the drug.

Seprase Targeted Neoantigens

Seprase is a serine protease that is very similar if not identical to FAP. The enzyme is over-expressed on the surface of malignant melanoma and breast cancer cells. The following references relate to this subject matter: Levy M.T., et al., "Fibroblast Activation Protein: a Cell Surface Dipeptidyl Peptidase and Gelatinase Expressed by Stellate Cells at the Tissue Remodelling Interface in Human Cirrhosis," *Hepatology,* 29(6):1768-78 (1999); Mueller S.C., et al., "A Novel Protease-docking Function of Integrin at Invadopodia," *J Biol Chem,* 35:24947-24952 (1999); Goldstein L.A., et al., "Molecular Cloning of Seprase: A Serine Integral Membrane Protease from Human Melanoma," *Biochem Biophys Acta,* 1361(1):11-9 (1997); Kelly T., "Evaluation of Seprase Activity," *Clin Exp Metastasis,* 17(1):57-62 (1999); Goldstein L.A.; Chen W.T., "Identification of an Alternatively Spliced Seprase mRNA that Encodes a Novel Intracellular Isoform," *J Biol Chem,* 275(4):2554-2559 (2000); Pineiro-Sanchez M.L., et al., "Identification of the 170-kDa Melanoma Membrane-Bound Gelatinase (Seprase) as a Serine Integral Membrane Protease," *J Biol Chem,*

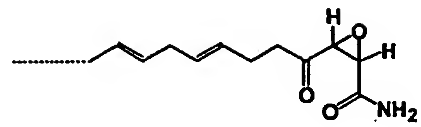
Alternatively Spliced Seprase mRNA that Encodes a Novel Intracellular Isoform," *J Biol Chem,* 275(4):2554-2559 (2000); Pineiro-Sanchez M.L., et al., "Identification of the 170-kDa Melanoma Membrane-Bound Gelatinase (Seprase) as a Serine Integral Membrane Protease," *J Biol Chem,* 272(12):7595-601 (1997); Mueller S.C., et al., "A Novel Protease-docking Function of Integrin at Invadopodia," *J Biol Chem,* 274(35):24947-24952 (1999); Monsky W.L., et al., "A Potential Marker Protease of Invasiveness, Seprase, is Localized on Invadopodia of Human Malignant Melanoma Cells," *Cancer Res,* 54(21):5702-10 (1994); Scanlan M.J., et al., "Molecular Cloning of Fibroblast Activation Protein Alpha, a Member of the Serine Protease Family Selectively Expressed in Stromal Fibroblasts Of Epithelial Cancers," *Proc Natl Acad Sci USA,* 91(12):5657-61 (1994); Goldstein L.A., et al., "Molecular Cloning of Seprase: A Serine Integral Membrane Protease From Human Melanoma," *Biochim Biophys Acta,* 1361(1):11-9 (1997); Kelly T., et al., "Seprase, a Membrane-Bound Protease, is Over-expressed by Invasive Ductal Carcinoma Cells of Human Breast Cancers," *Mod Pathol,* 11(9):855-63 (1998); Niedermeyer J., et al., "Mouse Fibroblast Activation Protein: Molecular Cloning, Alternative Splicing and Expression in the Reactive Stroma of Epithelial Cancers," *Int J cancer,* 71(3):383-9 (1997), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a mechanism based suicide inhibitor for seprase. Structures described above for FAP can be used to generate neoantigens to seprase.

Fatty Acid Synthetase Targeted Neoantigens.

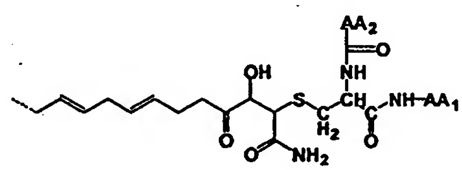
Fatty acid synthetase (FAS) is an enzyme, which catalyzes the synthesis of long chain fatty acids. The enzyme is over-expressed in breast cancer, colon cancer, ovarian, endometrial and prostate cancer. Inhibitors of FAS have been described as potential anti-cancer drugs, which trigger aptoptosis. Cerulenin is a mechanism based suicide inhibitor for FAS. A critical cysteine in the active site of the enzyme is alklated by cerulenin. This modification generates a neoantigen precursor, which can be used to trigger an immune response. The following references relate to this subject matter: Funabashi H., et al., "Binding Site of Cerulenin in Fatty Acid Synthetase," *J Biochem,* 105:751-755 (1989); Moche M., et al., "Structure of the Complex between the Antibiotic Cerulenin and Its Target, β-Ketoacyl-Acyl Carrier Protein Synthase," *J Biological Chem,* 274(10):6031-6034 (1999); Kuhajda F.P., et al., "Synthesis and Antitumor Activity of an Inhibitor of Fatty Acid Synthase," *Proc Natl Acad Sci USA,* 97(7):3450-3454 (2000); Pizer E.S., et al., "Pharmacological Inhibitors of Mammalian Fatty Acid Synthase Suppress DNA Replication and Induce Apoptosis in Tumor Cell Lines," *Cancer Res,* 58(20):4611-5 (1998); Pizer E.S., et al., "Malonyl-coenzyme-A is a Potential Mediator of Cytotoxicity Induced by Fatty-Acid Synthase Inhibition in Human Breast Cancer Cells and Xenografts," *Cancer Res,* 60(2):213-8 (2000); Gansler T.S., et al., "Increased Expression of Fatty Acid Synthase (OA-519) in Ovarian Neoplasms Predicts Shorter Survival," *Hum Pathol,* 28(6):686-92 (1997); Visca P., et al., "Immunohistochemical Expression of Fatty Acid Synthase, Apoptotic-Regulating Genes, Proliferating Factors, and Ras Protein Product in Colorectal Adenomas, Carcinomas, and Adjacent Nonneoplastic Mucosa," *Clin Cancer Res,* 5(12):4111-8 (1999); Kuhajda F.P.,

"Fatty-Acid Synthase and Human Cancer: New Perspectives on its Role in Tumor Biology," *Nutrition,* 16(3):202-208 (2000); Krontiras H., et al., "Fatty Acid Synthase Expression is Increased in Neoplastic Lesions of the Oral Tongue," *Head Neck,* 21(4):325-9 (1999); Nakamura I., et al., "Fatty Acid Synthase Expression in Japanese Breast Carcinoma Patients," *Int J Mol Med,* 4(4):381-7 (1999); Pizer E.S., et al., "Fatty Acid Synthase Expression in Endometrial Carcinoma: Correlation with Cell Proliferation and Hormone Receptors," *Cancer,* 83(3):528-37 (1998); Alo P.L., et al., "Fatty Acid Synthase (FAS) Predictive Strength in Poorly Differentiated Early Breast Carcinomas," *Tumori,* 85(1):35-40 (1999); Milgraum L.Z., et al., "Enzymes of the Fatty Acid Synthesis Pathway are Highly Expressed in In Situ Breast Carcinoma," *Clin Cancer Res,* 3(11):2115-20 (1997); Rashid A., et al., "Elevated Expression of Fatty Acid Synthase and Fatty Acid Synthetic Activity in Colorectal Neoplasia," *Am J Pathol,* 150(1):201-8 (1997); Jayakumar A., et al., "Human Fatty Acid Synthase: Properties and Molecular Cloning," *Proc Natl Acad Sci USA,* 92(19):8695-9 (1995); Hennigar R.A., et al., "Characterization of Fatty Acid Synthase in Cell Lines Derived from Experimental Mammary Tumors," *Biochim Biophys Acta,* 1392(1):85-100 (1998); Swinnen J.V., et al., "Androgens Stimulate Fatty Acid Synthase in the Human Prostate Cancer Cell Line LNCaP," *Cancer Res,* 57(6):1086-90 (1997); Kuhajda F.P., et al., "Fatty Acid Synthesis: A Potential Selective Target for Antineoplastic Therapy," *Proc Natl Acad Sci USA,* 91(14):6379-83 (1994); Kusakabe T., et al., "Fatty Acid Synthase is Expressed Mainly in Adult Hormone-sensitive Cells or Cells with High Lipid Metabolism and in Proliferating Fetal Cells," *J Histochem Cytochem,* 48:613-622 (2000), the contents of which are incorporated herein by reference in their entirety.



wherein the site of linker attachment to the rest of the drug is indicated by the dotted line.

The interaction of FAS and the above inhibitor can generate a neoantigen derived from FAS in which a cysteine of the enzyme is modified as shown below:



wherein AA1 and AA2 represent the amino acids adjacent to the modified cysteine residue. As in previous examples patients can be sensitized to the neoantigen by immunization with either appropriately modified FAS or by oligopeptides that correspond to the modified portion of the protein.

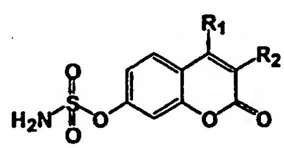
Steroid Sulfatase Targeted Neoantigens

Steroid sulfatase catalyzes the conversion of dehydroepiandrosterone sulfate and estrone sulfate into the unconjugated steroids. Steriod sulfatases are expressed in a variety of steriod dependent malignancies including breast cancer, ovarian, and endometrial cancer. Steroid sulfatase expression is an

independent risk factor for tumor recurrence in breast cancer patients. A variety of inhibitors to steroid sulfatase have been developed as potential therapies to suppress estrogen production and estrogen dependent malignancies. A variety of steroidal and nonsteroidal sulfamates have been described which are mechanism based suicide inhibitors of steroid sulfatase. The enzyme is covalently modified by sulfamoylation.

In a preferred embodiment, E is a mechanism based suicide inhibitor for steroid sulfatase. In a preferred embodiment, E is a sulfamate based suicide inhibitor of steroid sulfatase.

In a preferred embodiment (Eneo30), E is the following structure:



wherein R_1 and R_2 is a lower alkyl group, H, or a phenyl group; and wherein either R_1 or R_2 has a site for linker attachment to the remainder of the drug.

The neoantigen that results from the interaction of the inhibitor and steroid sulfatase can be a sulfamoylated enzyme. The pateint can be immunized either with this modified enzyme or with the corresponding sulfamolyated oligopeptide.

Epidermal Growth Factor Receptor Targeted Neoantigens

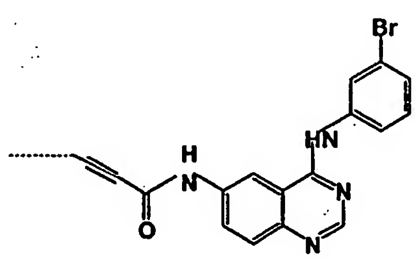
Epidermal growth factor receptors (EGFR) are membrane associated tyrosine kinases that are over-expressed in a large number of malignancies including: breast, prostate, ovarian, lung, gastric, and bladder. Aberrant activation of the tyrosine kinase activity results in neoplastic transformation. Accordingly, EGFR has attracted great attention as a target for anti-cancer therapy. Herceptin is a monoclonal antibody in clinical use for the treatment of breast cancer which binds to a member of the epidermal growth family (HER2) present on breast cancer cells. In patients with chemotherapy resistant metastatic HER2 + breast cancer treated with herceptin an objective response rate of 15% was observed. In hopes of improving therapy targeted to EGFR numerous inhibitors to EGFR have been developed. Unfortunately, inhibitors to EGFR tyrosine kinase are cytostatic rather than cytotoxic. The adenosine triphosphate binding site of EGFR has a reactive cysteine residue that is readily alkylated by a number of highly potent, selective irreversible inhibitors to EGFR. This covalent modification of the EGFR generates a neoantigen which can be exploited to target the immune system against EGFR + cancers resulting in tumor cell death rather than just growth suppression. The following references relate to this subject matter: Cobleigh M.A., et al., "Multinational Study of the Efficacy and Safety of Humanized Anti-HER2 Monoclonal Antibody in Women who have HER2-Overexpressing Metastatic Breast Cancer that has Progressed after Chemotherapy for Metastatic Disease," *J Clin Oncology,* 17(9):2639-2648 (1999); Discafani C.M., et al., "Irreversible Inhibition of Epidermal Growth Factor Receptor Tyrosine Kinase with *In Vivo* Activity by *N*-[4-[(3-Bromophenyl)amino]-6-quinazolinyl]-2-butynamide (CL-387,785)," *Biochem Pharm,* 57:917-925 (1999); Fry D.W., et al., "Specific, Irreversible Inactivation of the Epidermal

Growth Factor Receptor and erbB2, by a New Class of Tyrosine Kinase Inhibitor," *Proc Natl Acad Sci USA,* 95:12022-12027 (1998); Smaill J.B., et al., "Tyrosine Kinase Inhibitors. 4-(Phenylamino)quinazoline and 4-(Phenylamino)pyrido[*d*]pyrimidine Acrylamides as Irreversible Inhibitors of the ATP Binding Site of the Epidermal Growth Factor Receptor," *J Med Chem,* 42:1803-1815 (1999); Rewcastle G.W., et al., "Tyrosine Kinase Inhibitors. 10. Isomeric 4-[(3-Bromophenyl)amino]pyrido[*d*]-pyrimidines are Potent ATP Binding Site Inhibitors of the Tyrosine Kinase Function of the Epidermal Growth Factor Receptor," *J Med Chem,* 39:1823-1835 (1996); Rewcastle G.W., et al., "Tyrosine Kinase Inhibitors. 14. Structure-Activity Relationships for Methyl-amino-Substituted Derivatives of 4-[(3-Bromophenyl amino]-6-(methylamino)-pyrido[3,4-*d*]pyrimidine (PD 158780), a Potent and Specific Inhibitor of the Tyrosine Kinase Activity of Receptors for the EGF Family of Growth Factors," *J Med Chem,* 41:742-751 (1998); Bridges A.J., et al., "Tyrosine Kinase Inhibitors. 8. An Unusually Steep Structure-activity Relationship for Analogues of 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a Potent Inhibitor of the Epidermal Growth Factor Receptor," *J Med Chem,* 39:267-276 (1996); Thompson, A.M., et al., "Tyrosine Kinase Inhibitors. 13. Structure-Activity Relationships for Soluble 7-Substituted 4-[(3-Bromophenyl)amino]pyrido[4,3-*d*]pyrimidines Designed as Inhibitors of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor," *J Med Chem,* 40:3915-3925 (1997); Rewcastle G.W., et al., "Tyrosine Kinase Inhibitors. 9. Synthesis and Evaluation of Fused Tricyclic Quinazoline Analogues as ATP Site Inhibitors of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor," *J Med Chem,* 39:918-928 (1996); Rewcastle G.W., et al., "Tyrosine Kinase Inhibitors. 12. Synthesis

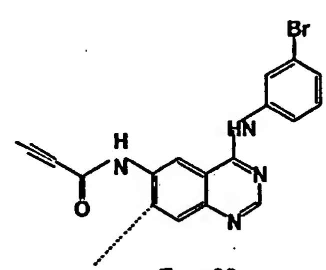
and Structure-Activity Relationships for 6-Substituted 4-(Phenylamino)pyrimido[5,4-*d*]pyrimidines Designed as Inhibitors of the Epidermal Growth Factor Receptor," *J Med Chem,* 40:1820-1826 (1997); Smaill J.B., et al., "Tyrosine Kinase Inhibitors. 17. Irreversible Inhibitors of the Epidermal Growth Factor Receptor: 4-(Phenylamino)quinazoline- and 4-(Phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides Bearing Additional Solubilizing Functions," *J Med Chem,* 43:1380-1397 (2000), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is an irreversible inhibitor to EGFR, which covalently modifies the protein generating a neoantigen.

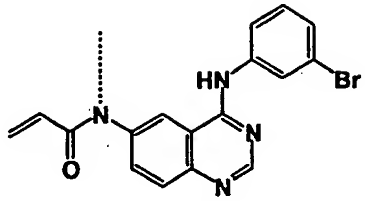
In preferred embodiments (Eneo31 to Eneo42), E comprises the following structures:



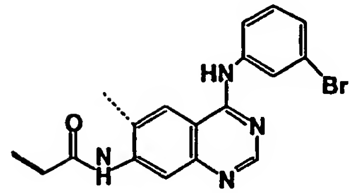
Eneo31



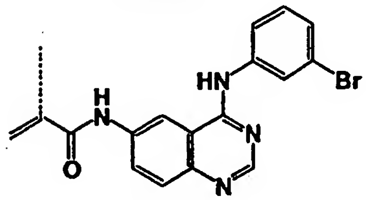
Eneo32



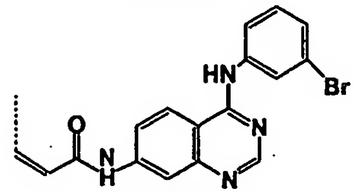
Eneo33



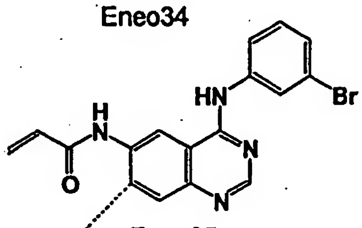
Eneo38



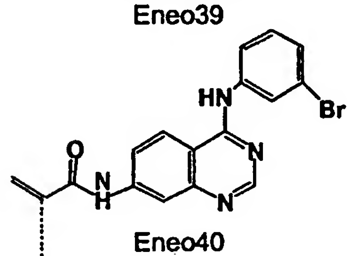
Eneo34



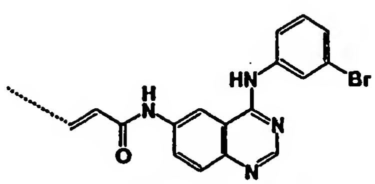
Eneo39



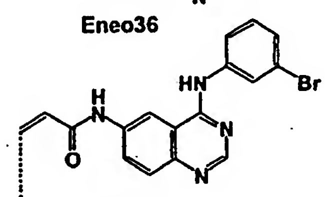
Eneo35



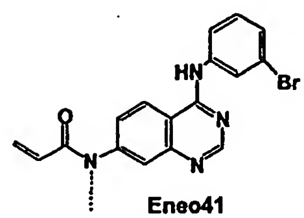
Eneo40



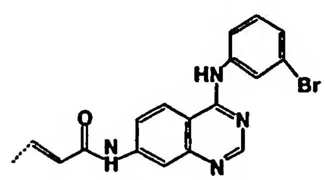
Eneo36



Eneo37



Eneo41



Eneo42

wherein the dotted line is the site of linker attachment to the remainder of the drug.

The neoantigens derived from the interaction of these inhibitors with EGFR can correspond to peptide sequences of the enzyme in which the thiol of cysteine 773 undergoes addition to the triple bond or the acrylamide double bond. Patients can be sensitized to these neoantigens either by immunization with the inhibited enzyme or by immunization with the corresponding modified oligopeptides neoantigens.

Phospatidylinositol 3-kinase Targeted Neoantigens

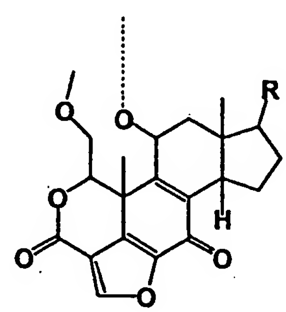
Phospatidylinositol 3-kinase (PIK3) is over-expressed in numerous malignancies including ovarian, breast, prostate, and lung cancer. The enzyme plays a key role in growth factor signal transduction. Over-expression results in neoplastic transformation. The PIK3CA oncogene, which is expressed in 40% of cases of ovarian cancer, encodes catalytic subunit of phosphatidylinositol 3-kinase. Accordingly, PIK3 is an attractive target for cancer therapy. A large number of inhibitors to the enzyme have been prepared as potential anti-cancer drugs. However, PIK3 inhibitors to date have exhibited toxicity and poor therapeutic index against tumors.

Wortmannin and related analogs are potent irreversible inhibitors of PIK3. The inhibitor covalently modifies the enzyme. The following references relate to this subject matter: Creemer L.C., et al., "Synthesis and *in Vitro* Evaluation of New Wortmannin Esters: Potent Inhibitors of Phosphatidylinositol 3-Kinase," *J Med Chem,* 39:5021-5024 (1996); Powis G., et al., "Wortmannin, a Potent and

Selective Inhibitor of Phosphatidylinositol-3-kinase," *Cancer Res*, 54:2419-2423 (1994); Norman B.H., et al., "Studies on the Mechanism of Phosphatidylinositol 3-Kinase Inhibition by Wortmannin and Related Analogs," *J Med Chem*, 39:1106-1111 (1996); Qiao L., et al., "3-Deoxy-D-*myo*-inositol 1-Phosphate, 1-Phosphonate, and Ether Lipid Analogues as Inhibitors of Phosphatidylinositol-3-kinase Signaling and Cancer Cell Growth," *J Med Chem*, 41(18):3303-3306 (1998); Vlahos C.J., et al., "A Specific Inhibitor of Phosphatidylinositol 3-Kinase, 2-(4-Morpholinyl))-8-phenyl-4H-1benzopyran-4-one (LY294002)," *J Biol Chem*, 269(7):5241-5248 (1994); Stefka Stoyanova et al, "Lipid Kinase and Protein Kinase Activities of G-Protein-Coupled Phosphoinositide 3-Kinase : Structure–Activity Analysis And Interactions with Wortmannin," *Biochem J*, 324, 489–495 (1997); Wymann M.P., et al "Wortmannin Inactivates Phosphoinositide 3-Kinase by Covalent Modification of Lys-802, a Residue Involved in the Phosphate Transfer Reaction," *Mol Cell Biol*, (4):1722-33 (1996), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is an irreversible inhibitor of PIK3.

In a preferred embodiment (Eneo43), E comprises the following structure:



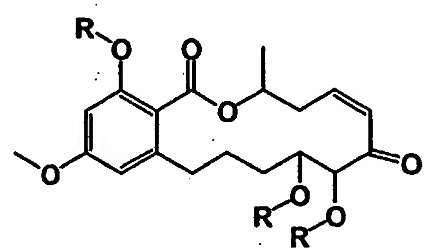
wherein the dotted line is the site of linker attachment to the remainder of ET and R is O, or OH.

Mitogen Activated Protein Kinase Kinase Targeted Neoantigens

Mitogen Activated Protein Kinase Kinase (MEK) plays a key role in growth factor signal transduction. Constitutive over-expression is oncogenic. Hyperactivity of the MEK-MAPK pathway is involved in numerous malignancies. Accordingly, MEK has attracted significant attention as a target for anti-cancer drugs. Inhibitors to MEK are cytostatic rather than cytotoxic and suppress tumor growth rather than killing tumors. Resorcylic acid lactones are extremely potent irreversible inhibitors of MEK. It is likely that the unsaturated alpha beta ketone alkylates a nucleophile in the active site of the enzyme. The covalently modified inhibited MEK can serve as a neoantigen for use in targeted immunotherapy. The following references relate to this subject matter: Zheng C.F; Guan K.L., "Cloning and Characterization of Two Distinct Human Extracellular Signal-regulated Kinase Activator Kinases, MEK1 and MEK2," *J Biol Chem,* 268(15):11435-9 (1993); Salh B., et al., "Investigation of the Mek-MAP Kinase-Rsk Pathway in Human Breast Cancer," *Anti-cancer Res,* 19(1B):731-40 (1999); Dudley D.T., et al., "A Synthetic Inhibitor of the Mitogen-Activated Protein Kinase Cascade," *Proc Natl Acad Sci USA,* 92:7686-7689 (1995); Sebolt-Leopold J.S., et al., "Blockade of the MAP Kinase Pathway Suppresses Growth of Colon Tumors *In Vivo,*" *Nature Med,* 5(7):810-816 (1999); Zhao A., et al., "Resorcylic Acid Lactones: Naturally Occurring Potent and Selective Inhibitors of MEK," *J Antibiotics,* 52(12):1086-1094 (1999); Hoshino R., et al., "Constitutive Activation of the 41-/43-kDa Mitogen-activated Protein Kinase Signaling Pathway in

Human Tumors," *Oncogene,* 18:813-822 (1999); Duesbery N.S., et al., "MEK Wars, a New Front in the Battle Against Cancer," *Nature Med,* 5(7):736-737 (1999), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is an irreversible inhibitor of MEK. In a preferred embodiment (Eneo44), E comprises the following structure:



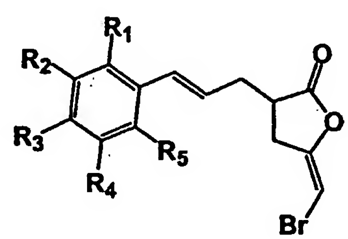
wherein R is H or the site of attachment to the remainder of the targeted drug by a trigger.

Glutathione S –Transferase Targeted Neoantigens

Glutathione S –Transferases (GST) are over-expressed by a variety of malignancies including ovarian, breast, renal, colon and lung cancer. GST can be massively over-expressed in chemotherapy resistant tumor cells. Haloenol lactones are mechanism based enzyme inhibitors of Pi type GST. The haloenol lactones covalently modify GST. In the process, a neoantigen is generated which can be exploited for targeted immunotherapy. The following references relate to this subject matter: Mitchell A.E., et al., "Structural and Functional Consequences of Haloenol Lactone Inactivation of Murine and Human

Glutathione *S*-Transferase," *Biochemistry,* 27:6752-6759 (1998); Zheng J., et al., "Haloenol Lactone is a New Isozyme-selective and Active Site-directed Inactivator of Glutathione *S*-Transferase," *J Biol Chem,* 271(34):20421-20425 (1996), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a mechanism based suicide inhibitor of GST. In a preferred embodiment, E is a haloenol lactone mechanism based inhibitor of GST. In a preferred embodiment (Eneo45), E comprises the following structure:



wherein R_1-R_5 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH or NO_2, an amino group, a cyano group, a carboxylate group, a phosphate, a phosphonate group, a sulfonate group, an ester group, or an amide group, and wherein either R_1, R_2, R_3, R_4, or R_5 has a site of attachment to the remainder of the target drug.

The neoantigen for immunization purposes can be prepared by treating the enzyme with an inhibitor based on the above structure.

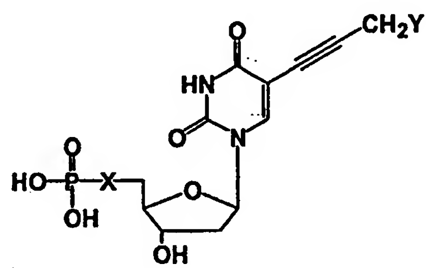
Thymidylate Synthase Targeted Neoantigens

Thymidylate synthase (TS) catalyzes the synthesis of conversion of deoxyuridine 5'-monophosphate into thymidine 5'-monophosphate. TS provides the sole de novo source of thymidylate, a key precursor for DNA synthesis. TS is over-expressed in a variety of malignancies including colorectal, and breast. TS has long been recognized as an important target in cancer therapy and is the basis of a number of anti-cancer drugs currently in clinical use. Clinical short comings of current TS inhibitors include toxicity, and the development of tumor resistance by over-expression of TS, thymidine kinase and the nucleoside transporter proteins.

A number of mechanism based suicide inhibitors of thymidylate synthase that covalently modify the enzyme are known. For example, 5-(3-fluoropropyn-1-yl)-2'-deoxyuridine 5' phosphate is a potent covalent inhibitor of TS. The interaction of TS and such an inhibitor can generate neoantigens, which can be exploited in targeted immunotherapy. The following references relate to this subject matter: Aschele C., et al., "Immunohistochemical Quantitation of Thymidylate Synthase Expression in Colorectal Cancer Metastases Predicts for Clinical Outcome to Fluórouracil-Based Chemotherapy," *J Clin Oncology,* 17(6):1760-1770 (1999); Lobo A.P., et al., "Mode of Action of Site-Directed Irreversible Folate Analogue Inhibitors of Thymidylate Synthase," *Biochem,* 37:4535-4542 (1998); Kalman T.I., et al., "Mechanism-Based Inactivation of Thymidylate Synthase by 5-(3-Fluoropropyn-1-yl)-2'-deoxyuridine 5'-Phosphate," *Biorg Med Chem Let,* 10:391-394 (2000); Bastian G., et al., "Inhibition of Thymidylate Synthetase by 5-Alkynyl-2'-deoxyuridylates," *J Med Chem,* 24:1385-1388 (1981); Montgomery

J.A., et al., "Phosphonate Analogue of 2'-deoxy-5-fluorouridylic Acid," *J Med Chem*, 22(1):109-11 (1979), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a mechanism based suicide inhibitor of TS. In a preferred embodiment (Eneo46), E comprises the following structure:



wherein X is O, CH_2, CHF, and CF_2, and Y is Cl, Br, F, I, or other good leaving group; and wherein E is attached to the remainder of ET by a biocleavable linker (a linker with a trigger) attached at either the phosphate, phosphonate, or hydroxy group.

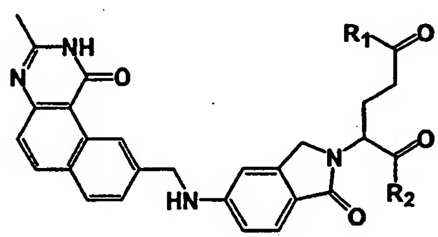
In a preferred embodiment, X is O or CH_2 and Y is F, and the site of attachment is at the phosphate or phosphonate group.

In another preferred embodiment, E is comprised of a ligand which binds to TS and to which is attached a free radical generator that can covalently modify TS and thereby generates neoantigens. 1843U89 is an extremely potent inhibitor of TS with a Ki of 90 pM. The following references relate to this subject matter:

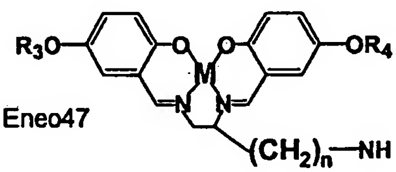
Duch D.S., et al., "Biochemical and Cellular Pharmacology of 1843U89, a Novel Benzoquinazoline Inhibitor of Thymidylate Synthase," *Cancer Res*, 53(4):810-8

(1993); Stout T.J.; Stroud R.M., "The Complex of the Anti-Cancer Therapeutic, BW1843U89, with Thymidylate Synthase at 2.0 a Resolution: Implications for a New Mode of Inhibition," *Structure,* 4(1):67-77 (1996), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (Eneo47 and Eneo48)), E comprises the following structures:

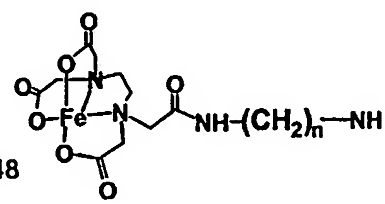


R1 or R2 =



Eneo47

R1 or R2 =

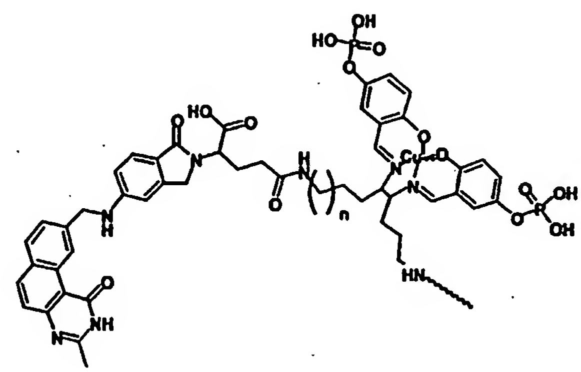


Eneo48

and wherein R_1 and R_2 can be OH or the structure as shown above, or the site of a trigger attachment; and wherein R3 and R4 are H , or bioreversible hydroxy masking groups, that can be converted in vivo into OH groups; and wherein n is 1-6, M is Cu (II) or Fe (II); and wherein E is linked to the remainder of ET by a trigger linked to one of the carboxylate or amino groups and wherein activation

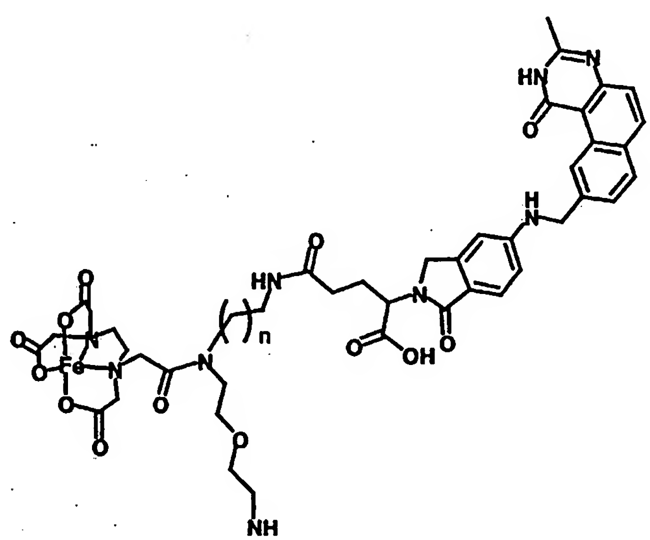
of said trigger liberates E from the remainder of ET. Suitable triggers have been described in the trigger section.

In a preferred embodiment (Eneo49) E comprises the following structure:



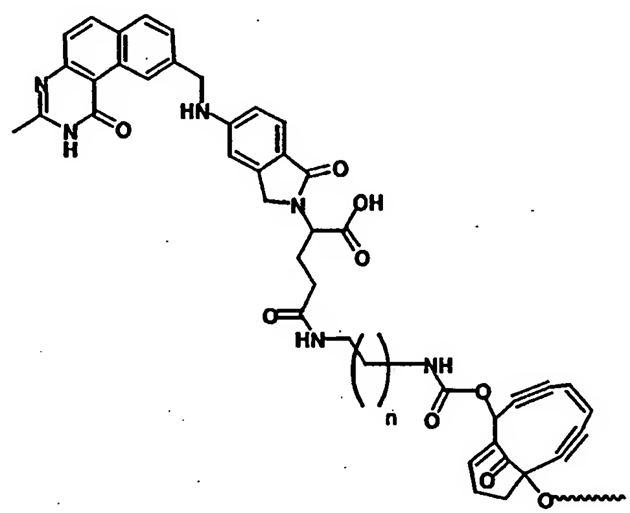
wherein n= 0,1,2,3,4,5,6,7, 8,9,10 or about 10; the cooper is Cu(II); and the wavy line is the site of attachment to the remainder of ET preferably the E is attached to a trigger that is activated inside cells.

In a preferred embodiment (Eneo50), E is comprised of the following structure:



wherein n= 0,1,2,3,4,5,6,7, 8,9,10 or about 10; the iron is Fe(II); and the wavy line is the site of attachment to the remainder of ET preferably the E is attached to a trigger that is activated inside cells.

In a preferred embodiment (Eneo 51) E is comprised of the following structure:



wherein n= 0,1,2,3,4,5,6,7, 8,9,10 or about 10; and the wavy line is the site of attachment to the remainder of ET preferably the E is attached to a trigger that is activated inside cells.

Cathepsin B Targeted Neoantigens

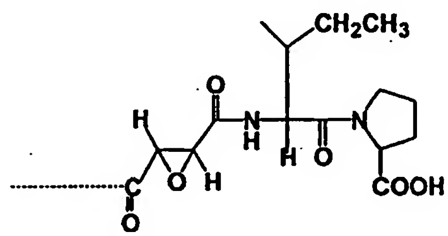
Cathepsin B (Cat B) is a cysteine protease, which is over-expressed in a large number of tumors including: lung, colon, prostate, breast, gastric, glioblastoma, thyroid, melanoma, and ovarian cancers. Cat B plays an important role in tissue invasion and angiogenesis. Cat B over-expression is associated with poor

patient outcome in a number of malignancies including: lung, brain, and breast cancers. A large number of inhibitors to Cat B have been developed. In addition, prodrugs designed to be activated by CAT B have been evaluated as anti-cancer drugs. However, to date there remains no satisfactory method to exploit Cat B as a tumor target. The following references relate to this subject matter: Foekens J.A., et al., "Prognostic Significance of Cathepsins B and L in Primary Human Breast Cancer," *J Clin Oncol,* 16:1013-1021 (1998); Yan S., et al., "Cathepsin B and Human Tumor Progression," *Biol Chem,* 379(2):113-23 (1998); Towatari T., et al., "Novel Epoxysuccinyl Peptides. A Selective Inhibitor of Cathepsin B, in Vivo," *FEBS,* 280(2):311-315 (1991); Matsumoto K., et al., "X-Ray Crystal Structure of Papain Complexed with Cathepsin B-specific Covalent-type Inhibitor: Substrate Specificity and Inhibitory Activity," *Biochim Biophys Acta,* 1383:93-100 (1998); Yamamoto A., et al., "Binding Mode of CA074, a Specific Irreversible Inhibitor, to Bovine Cathepsin B as Determined by X-Ray Crystal Analysis of the Complex," *J Biochem,* 121:974-977 (1997); Palmer, J.T., et al., "Vinyl Sulfones as Mechanism-Based Cysteine Protease Inhibitors," *J Med Chem,* 38:3193-3196 (1995), the contents of which are incorporated herein by reference in their entirety.

A large number of mechanism based suicide inhibitors of Cat B that colvalently modify the enzyme are known. The resulting neoantigen can be exploited in targeted immunotherapy.

In a preferred embodiment, E is a mechanism based suicide inhibitor of Cat B.

In a preferred embodiment (Eneo52), E comprises the following structure:



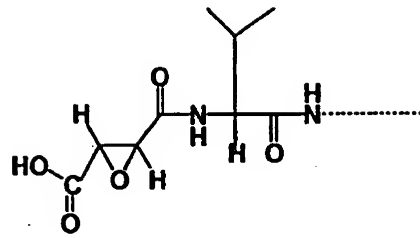
wherein the dotted line is the site of linker attachment to the remainder of the drug.

The resulting neoantigen is derived from the peptide of Cat B and the cysteine addition product to the epoxide ring.

Cathepsin L Targeted Neoantigens

Cathepsin L, like Cat B, is over-expressed by a number of malignancies. Mechansim based suicide inhibitors to Cat L are known and the resulting neoantigen can be exploited for targeted immunotherapy. The following references relate to this subject matter: Towatari T., et al., "Novel Epoxysuccinyl Peptides. A Selective Inhibitor of Cathepsin B, in Vivo," *FEBS*, 280(2):311-315 (1991), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a mechanism based suicide inhibitor or Cat L. In a preferred embodiment (Eneo53), E comprises the following structure:



wherein the dotted line is the site of linker attachment to the remainder of the drug.

Cathepsin K Targeted Neoantigens

Cathepsin K is over-expressed in a number of malignancies and can play a role in the mechanisms of development of metastatic bone lesions. Cat K is inhibited by compounds of similar structure as for Cat L and can be employed in neoantigen targeted immunotherapy in a similar fashion.

Ribonucleotide Diphosphate Reductase Targeted Neoantigens

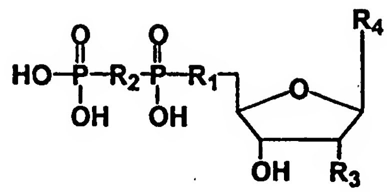
Ribonucleotide diphosphate reductase (RDPR) is a key enzyme in the synthesis of deoxyribonucleotides, which are essential precursors for DNA synthesis. RDPR is well recognized as a target of cancer therapy. Inhibition of the enzyme is central to the mechanism of action of a number of anti-cancer drugs including: hydroxyurea, Trimidox, (E)-2'-deoxy-2'-(fluoromethylene) cytidine, and gemcitabine. Current targeting of RDPR is associated with clinical toxicity and limited efficacy. Mechanism based suicide inhibitors that covalently modify

RDPR are known. The resulting neoantigen can be employed in targeted immunotherapy. The following references relate to this subject matter: Baker C.H. et al., "2'-Deoxy-2'-methylenecytidine and 2'-deoxy-2',2'-difluorocytidine 5'-diphosphates: Potent Mechanism-Based Inhibitors of Ribonucleotide Reductase," *J Med Chem,* 34(6):1879-84 (1991); Salowe S. et al., "Alternative Model for Mechanism-Based Inhibition of Escherichia Coli Ribonucleotide Reductase by 2'-azido-2'-deoxyuridine 5'-diphosphate," *Biochemistry,* 32(47):12749-60 (1993); Sjoberg B.M., et al., "A Substrate Radical Intermediate in the Reaction between Ribonucleotide Reductase from Escherichia Coli and 2'-Azido-2'-deoxynucleoside Diphosphates," *J Biol Chem,* 258(13):8060-7 (1983); Szekeres T., et al., "Biochemical and Antitumor Activity of Trimidox, a New Inhibitor of Ribonucleotide Reductase," *Cancer Chemother Pharmacol,* 34(1):63-6 (1994); Kang S.H.; Cho M.J., "Biological Activity and Phosphorylation of 2'-azido-2'-deoxyuridine and 2'-azido-2'-deoxycytidine," *Nucleosides Nucleotides,* 17(6):1077-88 (1998); Cory J.G., "Ribonucleotide Reductase as a Chemotherapeutic Target," *Adv Enzyme Regul,* 27:437-55 (1988); Bokemeyer C., et al., "Gemcitabine in Patients with Relapsed or Cisplatin-Refractory Testicular Cancer," *J Clin Oncol,* 17(2):512 (1999); van der Donk W.A., et al., "Inactivation of Ribonucleotide Reductase by (E)-2'-fluoromethylene-2'-deoxycytidine 5'-diphosphate: a Paradigm for Nucleotide Mechanism-Based Inhibitors," *Biochemistry,* 35(25):8381-91 (1996); Harris G. et al., "Mechanism of Inactivation of Escherichia Coli and Lactobacillus Leichmannii Ribonucleotide Reductases by 2'-chloro-2'-deoxynucleotides: Evidence for Generation of 2-methylene-3(2H)-furanone," *Biochemistry,* 23(22):5214-25 (1984); Masuda N., et al., "Phase I and Pharmacologic Study of Oral (E)-2'-deoxy-2'-

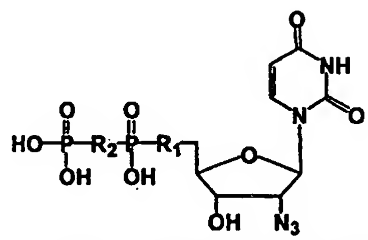
(fluoromethylene)cytidine: on a Daily x 5-day Schedule," *Invest New Drugs,* 16(3):245-54 (1998); Kang S.H. et al., "Synthesis and Biological Activity of bis(pivaloyloxymethyl) Ester of 2'-azido-2'-deoxyuridine 5'-monophosphate," *Nucleosides Nucleotides,* 17(6):1089-98 (1998); Szekeres T. et al., "The Enzyme Ribonucleotide Reductase: Target For Antitumor and Anti-HIV Therapy," *Crit Reve Clin Lab Sci,* 34(6):503-28 (1997); Takahashi T., et al., "Metabolism and Ribonucleotide Reductase Inhibition of (E)-2'-deoxy-2'-(fluoromethylene)cytidine, MDL 101, 731, in Human Cervical Carcinoma HeLa S3 Cells," *Cancer Chemother Pharmacol,* 41(4):268-74 (1998); Salowe S.P., et al., "Products of the Inactivation of Ribonucleoside Diphosphate Reductase from Escherichia coli with 2'-Azido-2'deoxyuridine 5'-Diphosphate," *Biochemistry,* 26:3408-3416 (1987); Thelander L., et al., "Active Site of Ribonucleoside Diphosphate Reductase from Escherichia Coli," *J Biological Chem,* 251(5):1398-1405 (1976); Bitonti A.J., et al., "Regression of Human Breast Tumor Xenografts in Response to (E)-2'deoxy-2'(fluoromethylene)cytidine, and Inhibitor of Ribonucleoside Diphosphate Reductase," *Cancer Res,* 54(6):1485-90 (1994); Bitonti A.J., et al., "Response of Human Colon and Prostate Tumor Xenografts to (E)-2'-deoxy-2'-(fluoromethylene) cytidine, an Inhibitor of Ribonucleotide Reductase," *Anti-cancer Res,* 15(4):1179-82 (1995), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a mechanism based suicide inhibitor of RDPR.

In a preferred embodiment (Eneo54), E comprises the following structure:



wherein R_1 and R_2 is O, CH_2, CHF, CF_2, and R_3 is azido, or a haolgen, and R_4 is a pyrimidine or purine base attached at N_1 or N_9 respectively; and wherein E is attached to the remainder of the targeted drug by a biocleavable linker (a linker with a trigger) attached at either the phosphate, phosphonate, or hydroxy group.



In a preferred embodiment (Eneo55), E comprises the following structure:

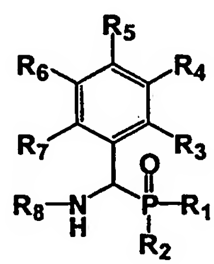
The interaction of RDPR with 2' azido, and 2' chloro nucleotide diphosphates covalently modifies the enzyme in an almost stochiometric mannner. There is evidence that the covalent modification is due to the generation of 2-methylene-3-(2H)–furanone in the active site. Regardless of the mechanism, the stable covalent modification of the enzyme can generate neoantigens that can be employed in targeted immunotherapy.

Trypsin Targeted Neoantigens

Trypsin is a serine protease, which is abnormally expressed by several important human malignancies including: ovarian cancer, gastric cancer, and lung cancer. A large number of mechanism based suicide inhibitors for trypsin are known and can be employed in neoantigen generation.

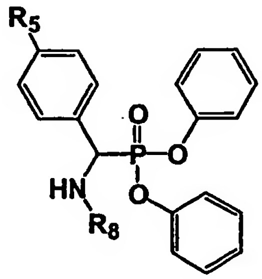
In a preferred embodiment, E is a mechanism based suicide inhibitor for trypsin.

In a preferred embodiment (Eneo56), E comprises the following structure:



wherein either R_1 or R_2 is a good leaving group for nucleophilic substitution reactions at phosphorus; and wherein R_3, R_4, R_6, and R_7 can be H, Cl, Br, F, I, , a lower alkyl group, a lower alkoxy group, OH, or NO_2; and wherein R_5 is an amidino group, a guanidino group, or a positively charged group, and R_8 is an oligopeptide or oligopeptide analog connected to the remainder of the drug.

In a preferred embodiment (Eneo57), E comprises the following structure:



wherein R_5 is an amidino or guanidino group.

Protein Kinase A Targeted Neoantigens

Protein Kinase A type 1 (PKA) or cyclic AMP dependent protein kinase is a serine/threonine kinase which is over-expressed in a wide range of malignancies including: breast, colon, prostate, melanoma, renal cell, and lung cancer. PKA is an intracellular enzyme and also is released by tumors into the extracellular space. The massive overproduction of PKA by tumors leads to an average 10-fold increase in PKA type 1 serum levels in cancer patients. Estrogen receptor negative breast cancer cells show even greater over-expression of PKA than hormone dependent cells. Activation of epidermal growth factor, which is central to many malignancies, is accompanied by increased expression of PKA. A variety of inhibitors have been developed as potential anti-cancer drugs targeted to PKA. The expression of PKA is not limited to tumor cells and to date no technology exists to effectively utilize PKA as an anti-cancer target. PKA over-expression can be utilized as a targeting variable in targeted neoantigen immunotherapy. The following references relate to this subject matter: Kondrashin A., et al., "Cyclic Adenosine 3':5'-Monophosphate-Dependent

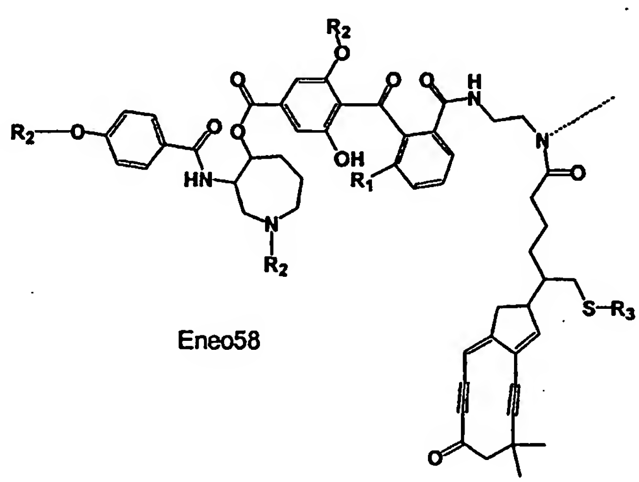
Protein Kinase on the External Surface of LS-174T Human Colon Carcinoma Cells," *Biochem,* 38(1):172-9 (1999); Putz T., et al., "Epidermal Growth Factor (EGF) Receptor Blockade Inhibits the Action of EGF, Insulin-Like Growth Factor I, and a Protein Kinase A Activator on the Mitogen-Activated Protein Kinase Pathway in Prostate Cancer Cell Lines," *Cancer Res,* 59(1):227-33 (1999); Nazareth L.V.; Weigel N.L., "Activation of the Human Androgen Receptor through a Protein Kinase A Signaling Pathway," *J Biol Chem,* 271(33):19900-19907 (1996); Ciardiello F., et al., "Antitumor Activity of Combined Blockade of Epidermal Growth Factor Receptor and Protein Kinase A," *J Natl Cancer Inst,* 88(23):1770-6 (1996); Ciardiello F.; Tortora G., "Interactions between the Epidermal Growth Factor Receptor and Type I Protein Kinase A: Biological Significance and Therapeutic Implications," *Clin Cancer Res,* 4(4):821-8 (1998); Ciardiello F., et al., "Down-Regulation of Type I Protein Kinase A by Transfection of Human Breast Cancer Cells with an Epidermal Growth Factor Receptor Antisense Expression Vector," *Breast Cancer Res Treat,* 47(1):57-62 (1998); Gordge P.C., et al., "Elevation of Protein Kinase A and Protein Kinase C Activities in Malignant as Compared with Normal Human Breast Tissue," *Eur J Cancer,* 32A(12):2120-6 (1996); Cho Y.S., et al., "Extracellular Protein Kinase A as a Cancer Biomarker: its Expression by Tumor Cells and Reversal by a Myristate-Lacking $C\alpha$ and RII^{β} Subunit Over-expression," *Proc Natl Acad Sci USA,* 97(2):835-840 (2000), the contents of which are incorporated herein by reference in their entirety.

Balanol and its analogs are potent reversible inhibitors of PKA and Protein Kinase C (PKC). The analog 10"-deoxybalanol inhibits PKA with a Ki of 4 nm

and PKC with a Ki of 640 nm. The following references relate to this subject matter: Gustafsson A.B., et al., "Differential and Selective Inhibition of Protein Kinase A and Protein Kinase C in Intact Cells by Balanol Congeners," *Molec Pharm,* 56:377-382 (1999); Narayana N., et al., "Crystal Structure of the Potent Natural Product Inhibitor Balanol in Complex with the Catalytic Subunit of cAMP-Dependent Protein Kinase," *Biochemistry*, 38(8):2367-2376 (1999); Setyawan J., et al., "Inhibition of Protein Kinases by Balanol: Specificity within the Serine/Threonine Protein Kinase Subfamily," *Mol Pharmacol,* 56(2):370-376 (1999), the contents of which are incorporated herein by reference in their entirety.

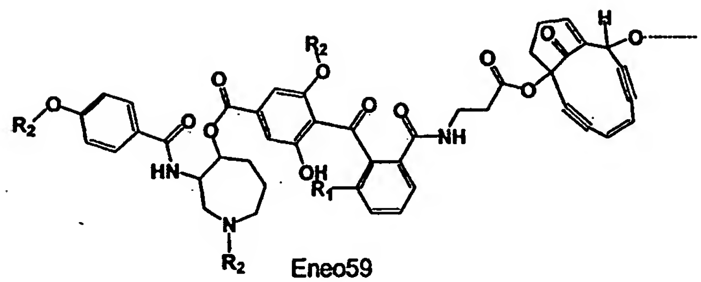
In a preferred embodiment, E is a ligand or a masked ligand for PKA to which is attached a triggerable free radical generator that irreversibly modifies PKA creating neoantigens.

In preferred embodiments (Eneo58 and Eneo59), E comprises the following structures:



Eneo58

or



Eneo59

wherein R_1 is H, or OH, the dotted line is the site of linker attachment to the remainder of ET; and R_2 is H or trigger, which functions as a masking group; and wherein R_3 is trigger, or a bioreversible thiol protecting group such as an acyl group or a –S-R_4 where R_4 is any group such that the resulting disulfide is converted in cells to the free thiol.

Dihydrofolate Reductase Targeted Neoantigens

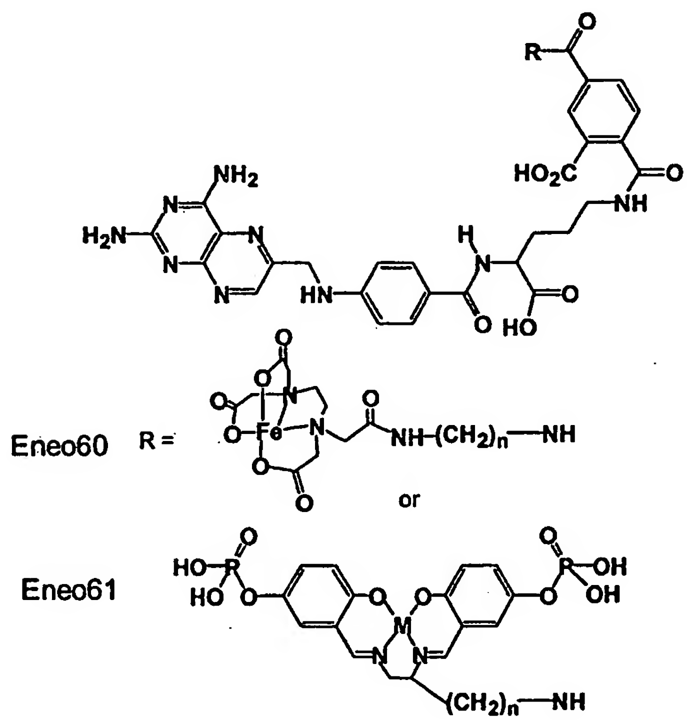
Dihydrofolate reductase (DHFR) catalyzes the reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate, which is essential for the synthesis of thymidylate, purines and glycine. DHFR is over-expressed in malignant cells and is under the control of the transcriptional factor E2F that plays a fundamental role in the biochemistry of malignancy. Inhibitors to DHFR such as methotrexate are routinely used as antineoplastic drugs. Gene amplification and over-expression of DHFR leads to resistance and impaired efficacy. In addition the metabolic block produced by inhibitors of DHFR can be by-passed by salvage pathways. The following references relate to this subject matter: Banerjee D., et al., "Molecular Mechanisms of Resistance to Antifolates, a Review," *Acta Biochem*

Pol, 42(4):457-64 (1995); Schuetz J.D., et al., "Transient Inhibition of DNA Synthesis By 5-Fluorodeoxyuridine Leads to Over-expression of Dihydrofolate Reductase with Increased Frequency of Methotrexate Resistance," *J Biol Chem,* 263(16):7708-12 (1988); Blakley R.L; Benkovic S.J., *Folates and Pterins*, John Wiley & Sons, New York 1984; Piper J.R., "Methotrexate and Related Diaminoheterocycles," in Caniam O. Foye , Ed., *Cancer Chemotherapeutic Agents*, American Chemical Society, Washington DC, 1995. p. 97; Eastman H.B., et al., "Stimulation of Dihydrofolate Reductase Promoter Activity by Antimetabolic Drugs," *Proc Natl Acad Sci USA,* 888:8572-8576 (1991); Müller H.; Helin K., "The E2F Transcription Factors: Key Regulators of Cell Proliferation," *Biochim Biophys Acta,* 1470:M1-M12 (2000), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is comprised of a ligand, which binds to DHFR to which is attached a triggerable free radical generator. A large number of inhibitors of DHFR that are suitable ligands are known. The x-ray structures of some DHFR inhibitor complexes are known and provide detailed information about the solvent accessible sites on inhibitors to which a linker and free radical generator can be attached without compromising binding affinity to the enzyme. The DHFR inhibitor PT523 binds with a Ki of 0.35pM. The following references relate to this subject matter: Rosowsky A., et al., "Analogues of N^{α}-(4-Amino-4-deoxypteroyl)-N^{δ}-hemiphthaloyl-L-ornithine (PT523) Modified in the Side Chain: Synthesis and Biological Evaluation," *J Med Chem,* 40:286-299 (1997); Johnson J.M., et al., "NMR Solution Structure of the Antitumor Compound PT523 and NADPH in the Ternary Complex with Human Dihydrofolate Reductase,"

Biochemistry, 36:4399-4411 (1997); Rosowsky A., et al., "Synthesis and Biological Activity of N^{ω}-Hemiphthaloyl-α,ω-diaminoalkanoic Acid Analogues of Aminopterin and 3',5-Dichloroaminopterin," *J Med Chem,* 37:2167-2174 (1994), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (Eneo60 and Eneo61), E comprises the following structures:



wherein n is 1-6, M is Cu (II) or Fe (II) and wherein E is linked to the remainder of ET by a trigger linked to one of the carboxylate or amino groups, which when activated, liberates E from the remainder of the drug. Suitable triggers have been described previously.

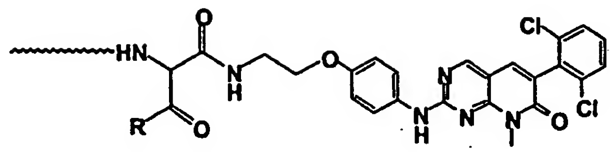
Src Kinase Targeted Neoantigens

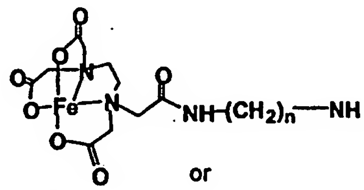
Src Kinases (Src) are a family of nonreceptor tyrosine kinases, which are over-expressed in a number of malignancies including: breast, pancreatic, colon cancer and myeloid leukemia. Constituitive over-expression of Src is oncogenic. Src has been recognized as a potential anti-cancer target; and a large number of inhibitors to Src have been developed. The following references relate to this subject matter: Verbeek B.S., et al., "c-Src Protein Expression is Increased in Human Breast Cancer. An Immunohistochemical and Biochemical Analysis," *J Pathol,* 180(4):383-8 (1996); Lutz M.P., et al., "Over-expression and Activation of the Tyrosine Kinase Src in Human Pancreatic Carcinoma," *Biochem Biophys Res Commun,* 243(2):503-8 (1998); Cartwright C.A., et al., "pp60c-src Activation in Human Colon Carcinoma," *J Clin Invest,* 83(6):2025-33 (1989); Brown M.T.; Cooper J.A., "Regulation, Substrates and Functions of Src," *Biochim Biophys Acta,* 1287(2-3):121-49 (1996); Rosen N., et al., "Analysis of pp60c-Src Protein Kinase Activity in Human Tumor Cell Lines and Tissues," *J Biol Chem,* 261(29):13754-9 (1986); Roginskaya V., et al., " Therapeutic Targeting of Src-Kinase Lyn in Myeloid Leukemic Cell Growth," *Leukemia,* 13(6):855-61 (1999); Moasser M.M., et al., "Inhibition of Src Kinases by a Selective Tyrosine Kinase Inhibitor Causes Mitotic Arrest," *Cancer Res,* 59(24):6145-52 (1999); Klutchko S.R., et al., "2-Substituted Aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-ones. Structure-Activity Relationships Against Selected Tyrosine Kinases and in Vitro and in Vivo Anti-cancer Activity," *J Med Chem,* 41:3276-3292 (1998); Hanke J.H., et al., "Discovery of a Novel, Potent, and Src Family-selective Tyrosine Kinase Inhibitor," *J Biol Chem,* 271(2):695-701 (1996); Panek R.L., et al., "*In Vitro* Pharmacological Characterization of PD 166285, a New Nanomolar Potent in

Broadly Active Protein Tyrosine Kinase Inhibitor," *J Pharm Exp Therap,* 283(3):1433-1444 (1997), the contents of which are incorporated herein by reference in their entirety.

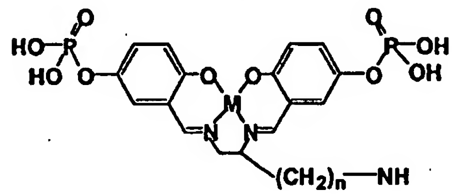
In a preferred embodiment, E is comprised of a ligand, which binds to Src and to which is attached a free radical generator. The generation of radicals can modify Src and create neoantigens. As mentioned above a large number of inhibitors, which bind tightly to Src, are known. Preferred embodiments are based on 2-Amino-8*H*-pyrido[2,3-*d*]pyrimidines analogs which bind at nanomloar levels to Src. The following references relate to this subject matter: Boschelli D.H., et al., "Synthesis and Tyrosine Kinase Inhibitory Activity of a Series of 2-Amino-8*H*-pyrido[2,3-*d*]pyrimidines: Identification of Potent, Selective Platelet-Derived Growth Factor Receptor Tyrosine Kinase Inhibitors," *J Med Chem,* 41:4365-4377 (1998), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (Eneo62 and Eneo63), E comprises the following structures:



Eneo62 R = 

or

Eneo63 

wherein n is 1-6, M is Cu (II) or Fe (II) and wherein E is linked to the remainder of the drug at the site indicated by the wavy line. In a preferred embodiment, E is linked to the remainder of the drug by a trigger, which is activated intracellularly and releases inside the cell the SRC binding free radical generator.

Platelet-derived Growth Factor Receptor Targeted Neoantigens

Platelet-derived growth factor receptors (PDGFR) are receptor tyrosine kinases which are over-expressed in numerous malignancies including ovarian, breast, prostate, pancreatic cancer, osteosarcoma, melanoma; and brain tumors. The constitutive expression of PDGFR tyrosine kinase activity is oncogenic. A large number of inhibitors to PDGFR tyrosine kinase have been developed as potential anti-cancer drugs. The following references relate to this subject matter: Coltrera M.D., et al., "Expression of Platelet-Derived Growth Factor B-Chain and the Platelet-Derived Growth Factor Receptor Beta Subunit in Human

Breast Tissue and Breast Carcinoma," *Cancer Res*, 55(12):2703-8 (1995); Henriksen R., et al., "Expression and Prognostic Significance of Platelet-Derived Growth Factor and its Receptors in Epithelial Ovarian Neoplasms," *Cancer Res*, 53(19):4550-4 (1993); Barnhill R.L., et al., "Expression of Platelet-Derived Growth Factor (PDGF)-A, PDGF-B and the PDGF-Alpha Receptor, but not the PDGF-Beta Receptor, in Human Malignant Melanoma in Vivo," *Br J Dermatol*, 135(6):898-904 (1996); Chott A., et al., "Tyrosine Kinases Expressed in Vivo by Human Prostate Cancer Bone Marrow Metastases and Loss of the Type 1 Insulin-Like Growth Factor Receptor," *Am J Pathol*, 155(4):1271-9 (1999); Ebert M., et al., "Induction of Platelet-Derived Growth Factor A and B Chains and Over-Expression of their Receptors in Human Pancreatic Cancer," *Int J Cancer*, 62(5):529-35 (1995); Shawver L.K, et al., "Inhibition of Platelet-Derived Growth Factor-Mediated Signal Transduction and Tumor Growth by N-[4-(trifluoromethyl)-phenyl]5-methylisoxazole-4-carboxamide," *Clin Cancer Res*, 3(7):1167-77 (1997); Bhardwaj B., et al., "Localization of Platelet-Derived Growth Factor Beta Receptor Expression in the Periepithelial Stroma of Human Breast Carcinoma," *Clin Cancer Res*, 2(4):773-82 (1996); Kawai T, et al., "Expression in Lung Carcinomas of Platelet-Derived Growth Factor and its Receptors," *Lab Invest*, 77(5):431-6 (1997); Oda Y., et al., "Expression of Growth Factors and their Receptors in Human Osteosarcomas. Immunohistochemical Detection of Epidermal Growth Factor, Platelet-Derived Growth Factor and their Receptors: Its Correlation with Proliferating Activities and P53 Expression," *Gen Diagn Pathol*, 141(2):97-103 (1995); Westermark B., et al., "Platelet-Derived Growth Factor in Human Glioma," *Glia*, 15(3):257-63 (1995); Liu Y.C., et al., "Platelet-Derived Growth Factor is an Autocrine

Stimulator for the Growth and Survival of Human Esophageal Carcinoma Cell Lines," *Exp Cell Res,* 228(2):206-11 (1996); Showalter H.D.; Kraker A.J., et al., "Small Molecule Inhibitors of the Platelet-Derived Growth Factor Receptor, the Fibroblast Growth Factor Receptor, and Src Family Tyrosine Kinases," *Pharmacol Ther,* 76(1-3):55-71 (1997); Palmer B. D., et al., " Structure-Activity Relationships for 5-Substituted 1-Phenylbenzimidazoles as Selective Inhibitors of the Platelet-Derived Growth Factor Receptor," *J Med Chem,* 42(13):2373-2382 (1999); Boschelli D.H., et al., "Synthesis and Tyrosine Kinase Inhibitory Activity of a Series of 2-Amino-8*H*-pyrido[2,3-*d*]pyrimidines: Identification of Potent, Selective Platelet-Derived Growth Factor Receptor Tyrosine Kinase Inhibitors," *J Med Chem,* 41(22):4365-4377 (1998), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E is a ligand that binds to PDGFR to which is attached a free radical generator which induces neoantigen formation. The structures shown above regarding Src targeted neoantigen formation can also target PDGFR and Fibroblast growth factor receptor which is similarly over-expressed in numerous malignancies and within the scope of this patent.

Estrogen Receptor Targeted Neoantigens

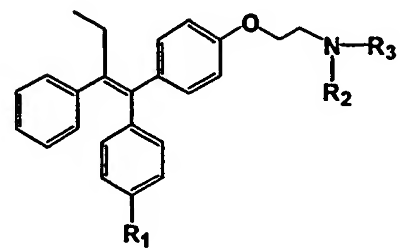
Estrogen receptors (ER) are over-expressed in a number of malignancies including breast cancer, ovarian, endometrial and some prostate cancers. Tamoxifen an antiestrogen is routinely used in the treatment of receptor positive breast cancer. Unfortunately approximately 50% of pateints with estrogen receptor positive breast cancer do not respond to tamoxifen. Resistance to

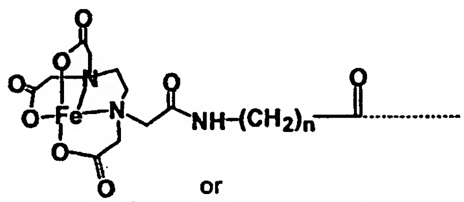
tamoxifen commonly develops despite the continued presence of the estrogen recptor. There have been a number of attempts to develop cytotoxins that are targeted towards the estrogen receptor. However there remains a need for a method to convert estrogen receptor expression into cytotoxicity. The following references relate to this subject matter: Macgregor J.I.; Jordan V.C., "Basic Guide to the Mechanisms of Antiestrogen Action," *Pharm Rev,* 50(2):151-196 (1998); Bonkhoff H., et al., "Estrogen Receptor Expression in Prostate Cancer and Premalignant Prostatic Lesions," *Am J Pathol,* 155:641-647 (1999); Devraj R., et al., "Design, Synthesis, and Biological Evaluation of Ellipticine-Estradiol Conjugates," *J Med Chem,* 39(17):3367-3374 (1996); Roger P., et al., "Increased Immunostaining of Fibulin-1, an Estrogen-Regulated Protein in the Stroma of Human Ovarian Epithelial Tumors," *Am J Pathol,* 153:1579-1588 (1998); Krohn K., et al., "Diethylstilbestrol-linked Cytotoxic Agents: Synthesis and Binding Affinity for Estrogen Receptors," *J Med Chem,* 32(7):1532-8 (1989); Zablocki J.A., et al., "Estrogenic Affinity Labels: Synthesis, Irreversible Receptor Binding, and Bioactivity of Aziridine-Substituted Hexestrol Derivatives," *J Med Chem,* 30(5):829-3; Leclercq G., "Guide-lines in the Design of New Antiestrogens and Cytotoxic-Linked Estrogens for the Treatment of Breast Cancer," *J Steroid Biochem,* 19(1A):75-85 (1983); Kohle H., et al., "Hexestrol-Linked Cytotoxic Agents: Synthesis and Binding Affinity for Estrogen Receptors," *J Med Chem,* 32(7):1538-47 (1989); Brinkman A., et al., "BCAR1, a Human Homologue of the Adapter Protein p130Cas, and Antiestrogen Resistance in Breast Cancer Cells," *J Nat Cancer Inst,* 92(2):112-120 (2000); V. Craig Jordan, "How is Tamoxifen's Action Subverted," *J Nat Cancer Inst,* 92(2):92-94 (2000); Rink S.M., et al., "Synthesis and Biological Activity of DNA Damaging Agents

that Form Decoy Binding Sites for the Estrogen Receptor," *Proc Natl Acad Sci*, 93:15063-15068 (1996); Kuduk S.D., et al., "Synthesis and Evaluation of Geldanamycin-Estradiol Hybrids," *Bioorg Med Chem Lett*, 9(9):1233-8 (1996); Robertson D.W., et al., "Tamoxifen Aziridines: Effective Inactivators of the Estrogen Receptor," *Endocrinology*, 109(4):1298-300 (1981), the contents of which are incorporated herein by reference in their entirety.

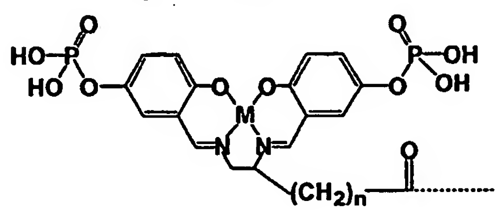
In a preferred embodiment, E is comprised of a ligand that binds to the estrogen receptors and to which is attached a moiety capable of irreversibly modifying the ER and generating neoantigens. A large number of compounds such as 4-hydroxy tamoxifen and raloxifene, bind estrogen receptors with high affinity and by known structural mechanisms. The following references relate to this subject matter: Macgregor J.I.; Jordan V.C., "Basic Guide to the Mechanisms of Antiestrogen Action," *Pharm Rev*, 50(2):151-196 (1998); Shiau A.K. et al., "The Structural Basis of Estrogen Receptor/Coactivator Recognition and the Antagonism of this Interaction by Tamoxifen," *Cell*, 95(7):927-37 (1998), the contents of which are incorporated herein by reference in their entirety.

In preferred embodiments (Eneo64 and Eneo65), E comprises the following structures:



Eneo64 R = 

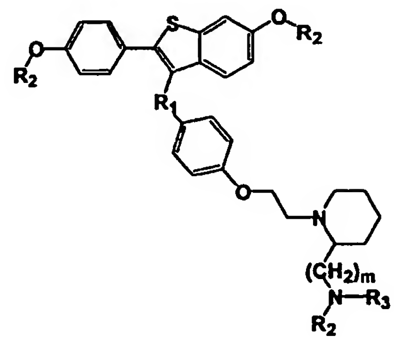
or

Eneo65 

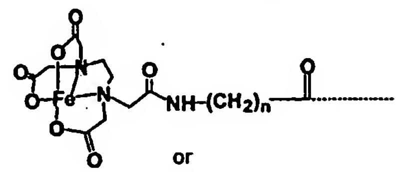
wherein R_1 is H, or OH, or the site of attachment of a trigger connected to the remainder of the targeted drug such that activation of the trigger liberates the tamoxifen analog, and wherein R_2 is H, methyl, or the site of attachment to the remainder of the targeted drug; and wherein n =1 to 6; and M is Cu(II) of Fe(II).

Another preferred set of structures is based on raloxifene. The following references relate to this subject matter: Palkowitz A.D., et al., "Discovery and Synthesis of [6-Hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]- 2-(4-hydroxyphenyl)]benzo[*b*]thiophene: A Novel, Highly Potent, Selective Estrogen Receptor Modulator," *J Med Chem,* 40(10):1407-1416 (1997), the contents of which are incorporated herein by reference in their entirety.

These preferred embodiments (Eneo66 and Eneo67) of E are shown below:

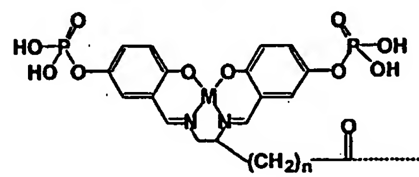


Eneo66
R3 =



or

Eneo67



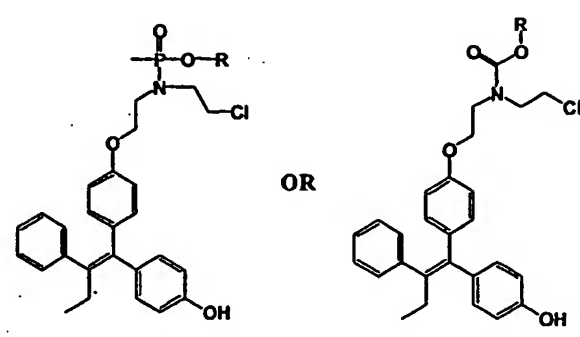
wherein R_1 is CO, CH_2, S, O, or NH, and m = 1 to 6; n=1,2,3,4,5,6 or about 6, M is Cu(II) or Fe(II); R2 is H, or the site of attachment of a trigger connected to the remainder of the targeted drug such that activation of the trigger liberates the raloxifene analog.

Other preferred embodiments are based on the ability of tamoxifen aziridine and related compounds to efficiently affinity label ER by alkylation of a cysteine residue. The following references relate to this subject matter: Katzenellenbogen J.A., et al., "Efficient and Highly Selective Covalent Labeling of the Estrogen Receptor with [^{3}H]Tamoxifen Aziridine," *J Biol Chem,* 258(6):3487-3495 (1983);

Harlow K.W., et al., "Identification of Cysteine 530 as the Covalent Attachment Site of an Affinity-labeling Estrogen (Ketononestrol Aziridine) and Antiestrogen (Tamoxifen Aziridine) in the Human Estrogen Receptor," *J Biol Chem,* 264(29):17476-17485 (1989); Reese J.C.; Katzenellenbogen B.S., "Mutagenesis of Cysteines in the Hormone Binding Domain of the Human Estrogen Receptor," 266(17):10880-10887 (1991); Aliau S., et al., "Cysteine 530 of the Human Estrogen Receptor α is the Main Covalent Attachment Site of 11*β*-(Aziridinylalkoxyphenyl)estradiols," *Biochemistry,* 38:14752-14762 (1999), the contents of which are incorporated herein by reference in their entirety.

In these embodiments, E is comprised of an ER binding ligand to which is coupled a latent alkylating agent which is unmasked upon activation of a trigger.

In a preferred embodiment (Eneo68 and Eneo69), E comprises the following structure:



Eneo 68 Eneo69

wherein R is a trigger attached to the remainder of the targeted drug such that activation of the trigger cleaves the phophoester or carbamate generating an

electrophilic species. A wide variety of suitable triggers have been described elsewhere in this patent.

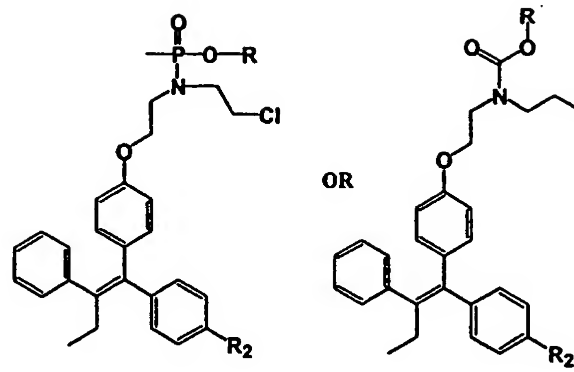
P-Glycoprotein Targeted Neoantigens

P-glycoprotein (p-G) is a protein that pumps a diverse range of drugs out of cells and is a major mediator of resistance to anti-cancer drugs. p-G is constitutively over-expressed in a large number of malignancies. In addition, p-G over-expression in human tumors may be rapidly induced by exposure to anti-cancer drugs. A large number of componds that inhibit p-G have been developed and some are in clinical trials as chemosensitzers. The following references relate to this matter: Ambudkar S.V., et al., "Biochemical, Cellular, and Pharmacological Aspects of the Multi-drug Transporter," *Annu Rev Pharmacol Toxicol* 39:361-398 (1999); and Sutoh I., et al., "Concurrent Expressions of Metallothionein, Glutathione S-transferase-pi, and P-glycoprotein in Colorectal Cancers," *Dis Colon Rectum,* 43(2):221-32 (2000); and Chan H.S., et al., "Immunohistochemical Detection of P-glycoprotein: Prognostic Correlation in Soft Tissue Sarcoma of Childhood," *J Clin Oncol,* 8:689-704 (1990); and Yang J.M., et al., "Inhibitory Effect of Alkylating Modulators on the Function of P-glycoprotein," *Oncol Res,* 9(9):477-84 (1997); and Callaghan R; Higgins C.F., "Interaction of Tamoxifen with the Multi-drug Resistance P-glycoprotein," *Br J Cancer,* 71(2):294-9 (1995); and Hofmann J., et al., "Mechanism of Action of Dexniguldipine-Hcl (B8509-035), A New Potent Modulator of Multi-drug Resistance," *Biochem Pharmacol,* 49(5):603-9 (1995); and Loo T.W; Clarke D.M., "Merck Frost Award Lecture 1998. Molecular Dissection of the Human Multi-drug Resistance P-glycoprotein," *Biochem Cell Biol,* 77(1):11-23 (1999); and Fracasso P.M., et al., "Phase I Study of Paclitaxel in Combination with a

Multi-drug Resistance Modulator, PSC 833 (Valspodar), in Refractory Malignancies," *J Clin Oncol,* 18(5):1124 (2000); and Tidefelt U., et al., "P-Glycoprotein Inhibitor Valspodar (PSC 833) Increases the Intracellular Concentrations of Daunorubicin In Vivo in Patients with P-Glycoprotein–Positive Acute Myeloid Leukemia," *J Clin Oncol,* 18(9):1837-1844 (2000); and Abolhoda A., et al., "Rapid Activation of MDR1 Gene Expression in Human Metastatic Sarcoma after In Vivo Exposure to Doxorubicin," *Clin Cancer Res,* 5(11):3352-6 (1999); and Traunecker H.C., et al., "The Acridonecarboxamide GF120918 Potently Reverses P-Glycoprotein-Mediated Resistance in Human Sarcoma MES-Dx5 Cells," *Br J Cancer,* 81(6):942-51 (1999); and Martin C., et al., "The Molecular Interaction of the High Affinity Reversal Agent XR9576 with P-glycoprotein," *Br J Pharmacol,* 128(2):403-11 (1999); and the contents are hereby incorporated by reference in their entirety.

In a preferred embodiment E is a ligand, which irreversibly modifies p-G and induces neoantigen formation. A large number of compounds are known which bind with high affinity to p-G. In a preferred embodiment, E is comprised of a p-G binding compound attached to an alkylating agent or a free radical generator. A preferred embodiment is based on the ability of tamoxifen aziridine to covalently bind to p-G. The following reference relates to this matter: Safa A.R., et al., "Tamoxifen Aziridine, a Novel Affinity Probe for P-glycoprotein in Multi-drug Resistant Cells," *Biochem Biophys Res Commun,* 202(1):606-12 (1994), and the contents are hereby incorporated by reference in their entirety. .

In a preferred embodiment(Eneo70),, E has the following structure:



wherein R is a trigger attached to the remainder of the targeted drug such that activation of the trigger cleaves the phophoester or carbamate generating an electrophilic species. A wide variety of suitable triggers have been described elsewhere in this patent. R_2 is H, OH, or O-CH_3.

Prostatic Acid Phosphatase Targeted Neoantigens

Prostatic acid phosphatase (PAP) is a marker for prostatic epithelial cells, which is expressed in prostate cancer. PAP has been recognized as a potential target for immunotherapy of prostate cancer. PAP is relatively nonspecific and is able to hydrolyse a broad range of phosphate esters including even large proteins with phosphorylated residues. The following references relate to this matter: Peshwa M.V., et al., "Induction of Prostate Tumor-Specific CD8+ Cytotoxic T-Lymphocytes in Vitro using Antigen-Presenting Cells Pulsed with Prostatic Acid Phosphatase Peptide," *Prostate,* 36(2):129-38 (1998); Ljung G., et al., "Characterization of Residual Tumor Cells Following Radical Radiation Therapy for Prostatic Adenocarcinoma; Immunohistochemical Expression of Prostate-Specific Antigen, Prostatic Acid Phosphatase, and Cytokeratin 8," *Prostate,* 31(2):91-7 (1997); Mori K.; Wakasugi C., "Immunocytochemical Demonstration

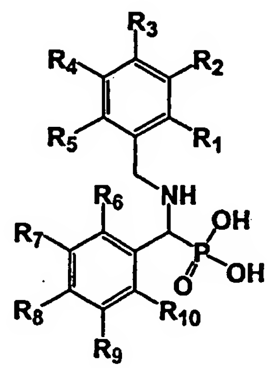
of Prostatic Acid Phosphatase: Different Secretion Kinetics between Normal, Hyperplastic and Neoplastic Prostates," *J Urol,* 133(5):877-83 (1985); Fong L., et al., "Induction of Tissue-Specific Autoimmune Prostatitis with Prostatic Acid Phosphatase Immunization: Implications for Immunotherapy of Prostate Cancer," *J Immunol,* 159(7):3113-7 (1997); Workman P., "Inhibition of Human Prostatic Tumour Acid Phosphatase by N,N-p-di-2-chloroethylaminophenol, N,N-p-di-2-chloroethylaminophenyl Phosphate and Other Difunctional Nitrogen Mustards," *Chem Biol Interact,* 20(1):103-12 (1978); Sinha A.A., et al., "Immunocytochemical Localization of an Immunoconjugate (Antibody IgG against Prostatic Acid Phosphatase Conjugated to 5-fluoro-2'-deoxyuridine) in Human Prostate Tumors," *Anti-cancer Res,* 18(3A):1385-92 (1998); Warhol M.J.; Longtine J.A., "The Ultrastructural Localization of Prostatic Specific Antigen and Prostatic Acid Phosphatase in Hyperplastic and Neoplastic Human Prostates," *J Urol,* 134(3):607-13 (1985); Lee H., et al., "Endogenous Protein Substrates for Prostatic Acid Phosphatase in Human Prostate," *Prostate,* 19(3):251-63 (1991); Lin M.F.; Clinton G.M., "Human Prostatic Acid Phosphatase has Phosphotyrosyl Protein Phosphatase Activity," *Biochem J,* 235(2):351-7 (1986); Wasylewska E., et al., "Phosphoprotein Phosphatase Activity of Human Prostate Acid Phosphatase," *Acta Biochim Pol,* 30(2):175-84 (1983); and the contents are hereby incorporated by reference in their entirety.

In a preferred embodiment E is a group which irreversibly modifies PAP and generates neoantigens. Benzylaminophosphonic acid derivatives inhibit PAP reversibly at nanomolar concentrations. The following reference relates to this matter: Beers S.A., et al., "Phosphatase Inhibitors--III. Benzylaminophosphonic

Acids as Potent Inhibitors of Human Prostatic Acid Phosphatase," *Bioorg Med Chem*, 4(10):1693-701 (1996), and the contents is hereby incorporated by reference in its entirety.

In a preferred embodiment E is comprised of a free radical generator coupled to an inhibitor of PAP.

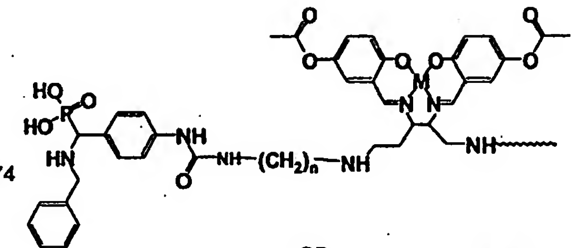
In a preferred embodiment (Eneo73) E is comprised of the structure shown below:



wherein a linker(s) coupled to a free radical generator and the remainder of the targeted drug is attached directly or indirectly to a site selected from R_1-to R_{10}, and wherein R_1-R_{10} may be inert groups which do not interfere with the binding to PAP. In preferred embodiments R_1-R_{10} are H, OH, a Cl, Br, F, I,, nitro, a phenol, a lower alkoxy group, an amino group, a lower alkyl group, $-CO_2H$, and --CO_2R_{11}; wherein R_{11} is a lower alkyl group; --$CONHR_{12}$; wherein NHR_{12} is an amino acid or oligopeptide.

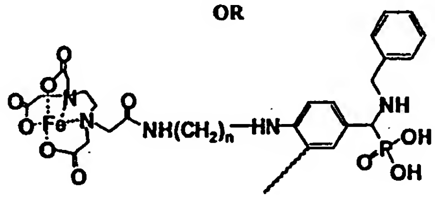
In preferred embodiments (Eneo74 and Eneo75), E is comprised of the structures shown below:

Eneo74



OR

Eneo75



wherein n=1,2,3,4,5,6 or about 6, M is Cu(II) or Fe(II), and the wavy line is the site of attachment to the remainder of the targeted drug.

Matrix Metalloprotease Targeted Neoantigens

Matrix metalloproteases are enzymes which degrade connective tissue and which are over-expressed by a large number of tumors and stroma of tumors. There have been an enormous number of inhibitors to matrix metalloproteases developed as potential anti-cancer drugs. However, inhibition of MMP activity does not typically produce cytotoxicity. At the present time there are no known methods to convert the over-expression of MMPs into selective tumor toxicity. The following reference relate to this matter: Nelson A.R., et al., "Matrix Metalloproteinases: Biologic Activity and Clinical Implications," *J Clin Oncol,* 18(5):1135 (2000); and Whittaker M., et al., "Design and Therapeutic Application

of Matrix Metalloproteinase Inhibitors," *Chem Rev,* 99:2735-2776 (1999); and Curran S.; Murray G.I., "Matrix Metalloproteinases in Tumour Invasion and Metastasis," *J Pathol,* 189(3):300-308 (1999); and the contents are hereby incorporated by reference in their entirety. .

In a preferred embodiment, E is a ligand, which binds to a matrix metalloprotease and irreversibly modifies the enzyme generating neoantigens.

Matrix Metalloproteinase 7 Targeted Neoantigens

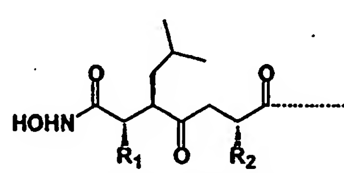
Matrix Metalloproteinase 7 (MMP-7 or Matrilysin) is a protease, which is constitutively produced by exocrine epithelial cells. MMP-7 is over-expressed by the tumor cells of a wide range of malignancies including ovarian, gastric, prostate, colorectal, endometrial, gliomas, and breast cancer. MMP-7 contrasts with many other metalloproteases which are over-expressed by tumor stromal elements rather then the tumor cells. At the present time there are no known methods to convert the over-expression of MMP-7 into selective tumor toxicity. The following references relate to this matter: Yamamoto H., et al., "Association of Matrilysin Expression with Recurrence and Poor Prognosis in Human Esophageal Squamous Cell Carcinoma," *Cancer Res,* 59(14):3313-6 (1999); Adachi Y., et al., "Contribution of Matrilysin (MMP-7) to the Metastatic Pathway of Human Colorectal Cancers," *Gut,* 45(2):252-8 (1999); Yamashita K, et al., "Expression and Tissue Localization of Matrix Metalloproteinase 7 (Matrilysin) in Human Gastric Carcinomas. Implications for Vessel Invasion and Metastasis," *Int J Cancer,* 79(2):187-94 (1998); Pacheco M.M., et al., "Expression of

Gelatinases A and B, Stromelysin-3 and Matrilysin Genes in Breast Carcinomas: Clinico-Pathological Correlations," *Clin Exp Metastasis,* 16(7):577-85 (1998); Hashimoto K., et al., "Expression of Matrix Metalloproteinase-7 and Tissue Inhibitor of Metalloproteinase-1 in Human Prostate," *J Urol,* 160(5):1872-6 (1998); Mori M., et al., "Over-expression of Matrix Metalloproteinase-7 mRNA in Human Colon Carcinomas," *Cancer,* 75(6 Suppl):1516-9 (1995); Honda M., et al., "Matrix Metalloproteinase-7 Expression in Gastric Carcinoma," *Gut,* 39(3):444-8 (1996); Nakano A., et al., "[Increased Expression of Gelatinases A and B, Matrilysin and TIMP-1 Genes in Human Malignant Gliomas]," *Nippon Rinsho,* 53(7):1816-21 (1995); Knox J.D., et al., "Matrilysin Expression in Human Prostate Carcinoma," *Mol Carcinog,* 15(1):57-63 (1996); Adachi Y., et al., "Matrix Metalloproteinase Matrilysin (MMP-7) Participates in the Progression of Human Gastric and Esophageal Cancers," *Int J Oncol,* 13(5):1031-5 (1998); Ueno H., et al., "Enhanced Production and Activation of Matrix Metalloproteinase-7 (Matrilysin) in Human Endometrial Carcinomas," *Int J Cancer,* 84(5):470-7 (1999); Barille S., et al., "Production of Metalloproteinase-7 (Matrilysin) by Human Myeloma Cells and its Potential Involvement in Metalloproteinase-2 Activation," *J Immunol,* 163(10):5723-8 (1999); Senota A., et al.," Relation of Matrilysin Messenger RNA Expression with Invasive Activity in Human Gastric Cancer," *Clin Exp Metastasis,* 16(4):313-21 (1998); Saarialho-Kere U.K., et al., "Matrix Metalloproteinase Matrilysin is Constitutively Expressed in Adult Human Exocrine Epithelium," *J Invest Dermatol,* 105(2):190-6 (1995);. Tanimoto H., et al., "The Matrix Metalloprotease Pump-1 (MMP-7, Matrilysin): A Candidate Marker/Target for Ovarian Cancer Detection and Treatment," *Tumour*

Biol, 20(2):88-98 (1999); and the contents are hereby incorporated by reference in their entirety.

In a preferred embodiment, E is a ligand for MMP-7 to which is attached a triggerable free radical generator. A large number of potent reversible ligands are known that reversibly inhibit MMP-7. These ligands will tether the free radical generator to MMP-7 and focus free radical induced protein modification leading to the generation of MMP-7 based neoantigens. The following references relate to this matter: Pratt L.M., et al., "The Synthesis of Novel Matrix Metalloproteinase Inhibitors Employing the Ireland-Claisen Rearrangement," *Bioorg Med Chem Lett*, 8:1359-1364 (1998); and Abramson S.R., et al., "Characterization of Rat Uterine Matrilysin and Its cDNA," *J Biological Chem*, 270(27):16016-16022 (1995); and Nelson A.R., et al., "Matrix Metalloproteinases: Biologic Activity and Clinical Implications," *J Clin Oncology*, 18(5):1135-1149 (2000); and Whittaker M., et al., "Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors," *Chem Rev*, 99:2735-2776 (1999) and the contents are hereby incorporated by reference in their entirety.

In a preferred embodiment, E is comprised of a MMP-7 ligand of the following structure:

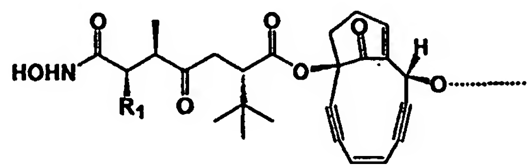


wherein the dotted line is the site of attachment or linker attachment to the triggerable free radical generator and wherein R_1 is hydroxy, methyl, ethyl,

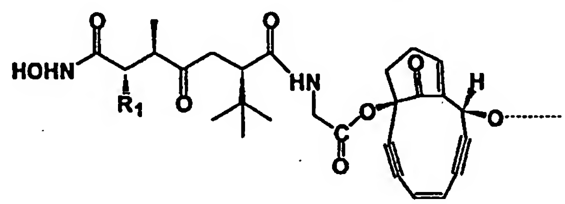
isopropyl, cyclopentyl, 3-(tetrahydrothiophenyl), or thiopen-2-ylthiomethyl; and wherein R_2 is benzyl, t-butyl, or isopropyl.

In preferred embodiments (Eneo76-Eneo79), E has the following structures:

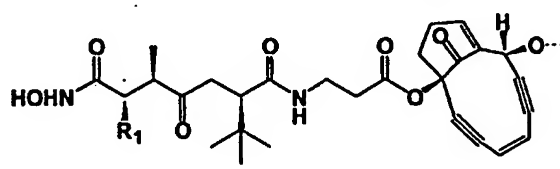
Eneo76



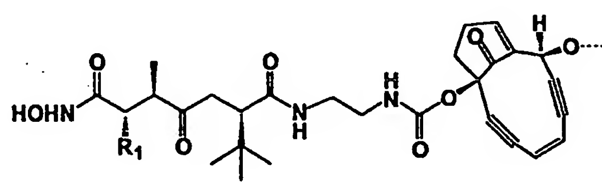
Eneo77



Eneo78



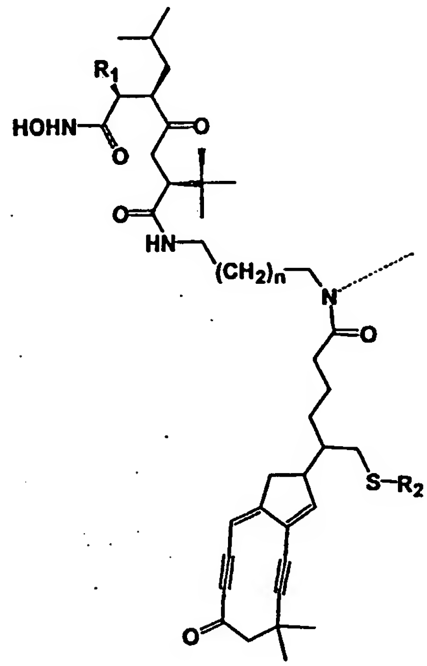
Eneo79



and wherein, the dotted line indicates the site of attachment of the remainder of the drug.

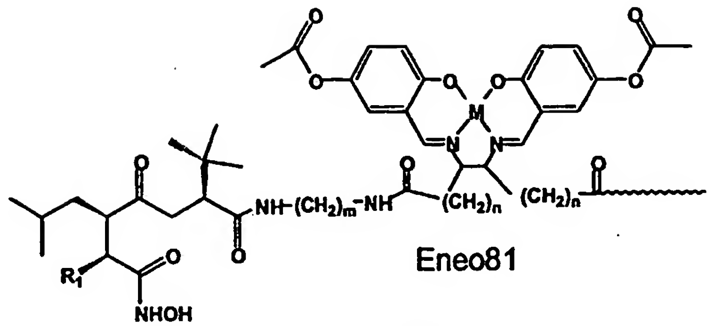
As discussed previously, addition of a thiol to the double bond of the free radical generator will trigger the formation of a reactive diradical that will react with the MMP-7 and generate neoantigens.

In another preferred embodiment (Eneo80), E has the following structure:



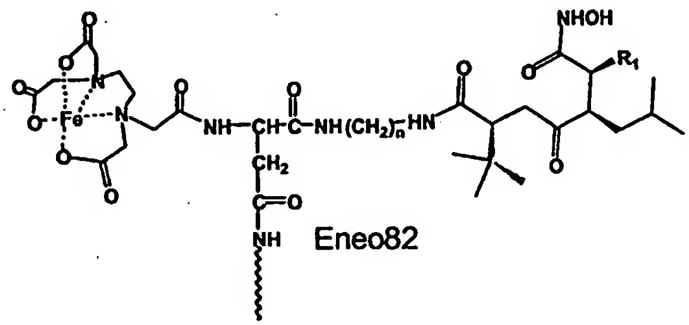
wherein the dotted line is the site of linker attachment to the remainder of ET and wherein R_1 is hydroxy, methyl, ethyl, isopropyl, cyclopentyl, 3-(tetrahydrothiophenyl), or thiopen-2-ylthiomethyl-; and wherein R_2 is an acyl group, or R_2 is a clock-like time delay trigger, or a bioreversible thiol protecting group such as –S-R_4, where R_4 is a group such that the disulfide is reduced to the thiol by cells; and wherein N=0,1,2,3,4,5,6 or about 6.

In other preferred embodiments (Eneo81 and Eneo82), E has the following structures:



Eneo81

OR



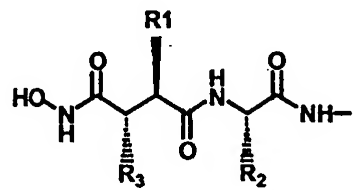
Eneo82

wherein n =1,2,3,4,5,6 or about 6; m=2,3,4,5,6 or about 6; the wavy line is the site of linker attachment to the remainder of ET; and wherein R_1 is hydroxy, methyl, ethyl, isopropyl, cyclopentyl, 3-(tetrahydrothiophenyl), or thiopen-2-ylthiomethyl; and wherein M is Cu(II) or Fe(II).

Neoantigen Formation Targeted to MMP1, 2, 3, 9 and Membrane Type 1 MMP.

MMP 1, 2, 3, 9 and membrane type MMP 1(MT-MMP-1) are all over-expressed in a wide variety of malignancies.

Similarities in the active site of these enzymes allow for targeting with a common family of ligands. The neoantigens generated and required for sensitization however should be unique for each enzyme. Compounds of the following structure bind reversibly to MMP 1, 2, 3, 9 and membrane type MMP 1 with IC_{50}

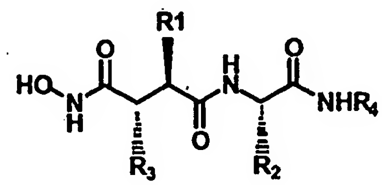


in the nanomolar to subnanomolar range.

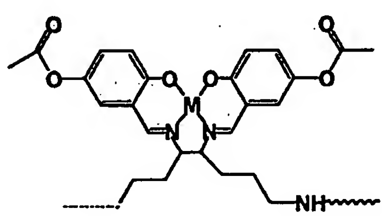
wherein R_1 is $-CH_2CH(CH_3)_2$, $-(CH_2)_2C_6H_5$, $-(CH_2)_3C_6H_5$, n-butyl, n-hexyl, or n-octyl; R2 is C_6H_5, — C_6H_{11}, - $C(CH_3)_3$, (indol-3-yl)methyl, $-CH_2C_6H_5$, (5, 6, 7, 8,-terahydro-1-napthyl)methyl, $-CH(CH_3)_2$, 1-(napthyl)methyl, 3-(napthyl)methyl, 1-(quinolyl)methyl, 3-(quinolyl)methyl, 3-pyridylmethyl, 4-pyridylmethyl, or t-butyl; and R3 is H, OH, methyl, 2-thienylthiomethyl, or allyl.

The following references relate to this matter:Yamamoto M., et al., "Inhibition of Membrane-Type 1 Matrix Metalloproteinase by Hydroxamate Inhibitors: An Examination of the Subsite Pocket," *J Med Chem*, 41:1209-1217 (1998).;and Curtin M.L., et al., "Broad Spectrum Matrix Metalloproteinase Inhibitors: An Examination of Succinamide Hydroxamate Inhibitors with P_1C_α Gem-Disubstitution," *Biorg Med Chem Lett*, 8:1443-1448 (1998); and Levy D.E., et al., "Matrix Metalloproteinase Inhibitors: A Structure-Activity Study," *J Med Chem*, 41:199-223 (1998) and their contents are hereby incorporated by reference in their entirety. .

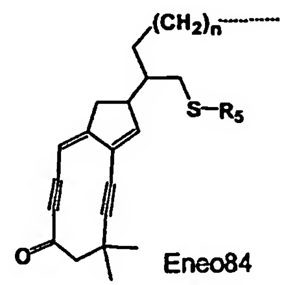
In a preferred embodiment, E is comprised of a ligand which binds to MMP1, 2, 3, 9 or MT-MMP-1 to which is attached a free radical generator. In preferred embodiments (Eneo8- Eneo86), E has the following structures:



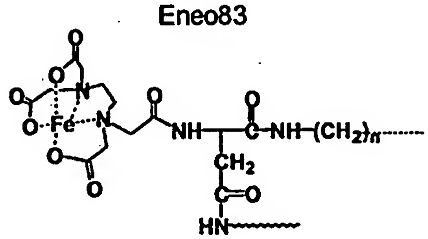
wherein R_4 has the following structure:



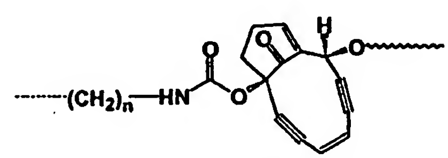
Eneo83



Eneo84



Eneo85

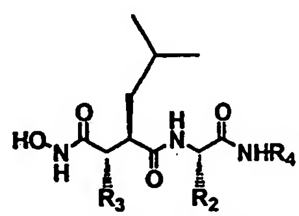


Eneo86

and wherein the dotted line is the site of attachment to the N of the MMP ligand the wavy line is the site of attachment to the remainder of the targeted drug ; and wherein R_1 is $-CH_2CH(CH_3)_2$, $-(CH_2)_2C_6H_5$, $-(CH_2)_3C_6H_5$, n-butyl, n-hexyl, or n-octyl. R_2 is C_6H_5, — C_6H_{11}, - $C(CH_3)_3$, (indol-3-yl)methyl, $-CH_2C_6H_5$, (5, 6,

7, 8, -terahydro-1-napthyl)methyl, $-CH(CH_3)_2$; 1-(napthyl)methyl, 3-(napthyl)methyl, 1-(quinolyl)methyl, 3-(quinolyl)methyl, 3-pyridylmethyl, 4-pyridylmethyl, or t-butyl; and R_3 is H, OH, methyl, 2-thienylthiomethyl, or allyl; and wherein n=1,2,3,4,5,6 or about 6 and M is Cu(II) of Fe(II); and wherein R_5 is an acyl group, or R_5 is a clock-like time delay trigger, or a bioreversible thiol protecting group such as $-S-R_6$; where R_6 is any group such that the disulfide is reduced to the thiol by cells.

In preferred embodiments (Eneo87), E has the following structures:



wherein R_2 is benzyl and R_3 is 2-thienylthiomethyl; or wherein R_2 is 5, 6, 7, 8, -terahydro-1-napthyl)methyl, and R_3 is methyl; or wherein R_2 is t-butyl and R_3 is OH; or wherein R_2 is H and R_3 is (indol-3-yl)methyl; and wherein R_4 is as shown above.

Another preferred embodiment is based on diphenlyether sulfone inhibitors of MMP's which are highly active against MMP2, 3, 9, 12, and 13. The following references relate to this matter: 5,932,595, 8/03/99 Bender et al., "Matrix Metalloprotease Inhibitors"; and Lovejoy B., et al., "Crystal Structures of MMP-1 and -13 Reveal the Structural Basis for Selectivity of Collagenase Inhibitors," *Nat Struct Biol,* 6(3):217-21 (1999)and; Botos I., et al., "Structure of

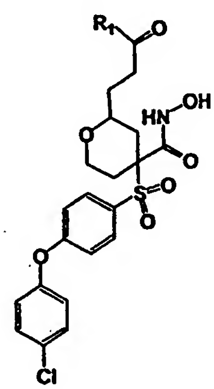
Recombinant Mouse Collagenase-3 (MMP-13)," *J Mol Biol*, 292:837-844 (1999), and their contents are hereby incorporated by reference in their entirety.

MMP 13 is an attractive target for neoantigen formation as it is over-expressed in a wide range of malignancies.

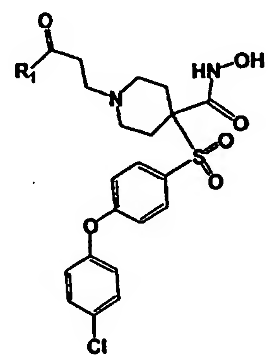
Pendas A.M., et al., "An Overview of Collagenase-3 Expression in Malignant Tumors and Analysis of its Potential Value as a Target in Antitumor Therapies," *Clin Chim Acta*, 291(2):137-55 (2000); and Shalinsky D.R., et al., "Broad Antitumor and Antiangiogenic Activities of AG3340, a Potent and Selective MMP Inhibitor Undergoing Advanced Oncology Clinical Trials," *Ann NY Acad Sci*, 878:236-70 (1999); and Johansson N., et al., "Collagenase-3 (MMP-13) is Expressed by Tumor Cells in Invasive Vulvar Squamous Cell Carcinomas," *Am J Pathol*, 154(2):469-80 (1999); and Barmina O.Y., et al., "Collagenase-3 Binds to a Specific Receptor and Requires the Low Density Lipoprotein Receptor-Related Protein for Internalization," *J Biol Chem*, 274(42):30087-93 (1999); and Cazorla M., et al., "Collagenase-3 Expression is Associated with Advanced Local Invasion in Human Squamous Cell Carcinomas of the Larynx," *J Pathol*, 186(2):144-150 (1998); and Balbin M., et al., "Expression and Regulation of Collagenase-3 (MMP-13) in Human Malignant Tumors," *APMIS*, 107(1):45-53 (1999); and Johansson N., et al., "Expression of Collagenase-3 (Matrix Metalloproteinase-13) in Squamous Cell Carcinomas of the Head and Neck," *Am J Pathol*, 151(2):499-508 (1997); and Uria J.A., et al., "Regulation of Collagenase-3 Expression in Human Breast Carcinomas is Mediated by Stromal-Epithelial Cell Interactions," *Cancer Res*, 57(21):4882-8 (1997); and

Airola K., et al., "Human Collagenase-3 is Expressed in Malignant Squamous Epithelium of the Skin," *J Invest Dermatol,* 109:225-231 (1997); and Freije J.M., et al., "Molecular Cloning and Expression of Collagenase-3, A Novel Human Matrix Metalloproteinase Produced by Breast Carcinomas," *J Biol Chem,* 269(24):16766-73 (1994); and Uria J.A., et al., "Regulation of Collagenase-3 Expression in Human Breast Carcinomas is Mediated by Stromal-Epithelial Cell Interactions," *Cancer Res,* 57(2):4882-8 (1997); and their contents are hereby incorporated by reference in their entirety.

In preferred embodiments (Eneo87 and Eneo88) E has the following structure:

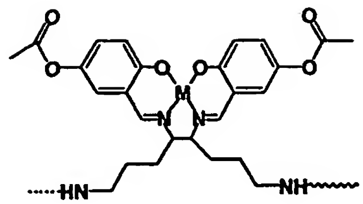


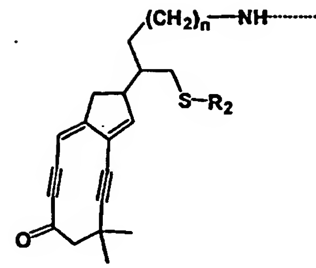
Eneo87

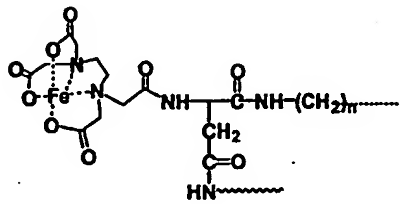


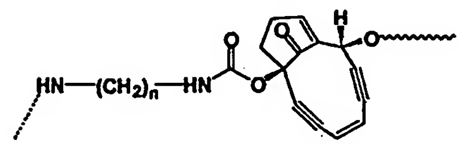
Eneo88

wherein R_1 is as shown below:









and wherein the dotted line is the site of attachment to the MMP ligand the wavy line is the site of attachment to the remainder of the targeted drug; and wherein n=1,2,3,4,5,6 or about 6, and M is Cu(II) of Fe(II); and wherein R_2 is an acyl group, or R_2 is a clock-like time delay trigger, or a bioreversible thiol protecting group such as –S-R_3 where R_3 is any group such that the disulfide is reduced to the thiol by cells.

Targeted Delivery of Activators of Innate Immunity

Evolution has endowed the body with the ability to mount effective and almost immediate nonspecific defenses against infectious agents. The body is highly tuned to detect and react to molecules derived from pathogens. The result is a rapid and massive influx of inflammatory cells such as neutrophils, monocyes, macrophages, natural killer cells and delta/gamma T cells. The release of a number of inflammatory cytokines amplifies the response. Phagocytosis and the

production of toxic radicals such as superoxide, hypochlorous acid, nitric oxide, and peroxynitrite contribute to the killing of the invading microorganisms. The same intense immune response that is ellicited by microorganisms can be directed against tumors by selective targeting of activators of innate immunity to tumors. The ability of activated neutrophils, macrophages, monocytes and NK cells to kill tumors is well documented in numerous models. A pronounced synergy is expected when tumors are simultaneously targeted with both activators of innate immunity and antigen receptor specific T cell mediated immunity. The immune system evolved to deal precisely with this situation. Both innate and adaptive immune responses are simultaneously triggered by infectious agents and mutually reinforce and amplify the net immune response.

The following references relate to this matter: Seino K., et al., "Antitumor Effect of Locally Produced CD95 Ligand," *Nat Med*, 3(2):165-70 (1997); and Shimizu M., et al., "Induction of Antitumor Immunity with Fas/APO-1 Ligand (CD95L)-Transfected Neuroblastoma Neuro-2a Cells," *J Immunol*, 162(12):7350-7 (1999); and Stoppacciaro A., et al., "Regression of an Established Tumor Genetically Modified to Release Granulocyte Colony-stimulating Factor Requires Granulocyte-T Cell Cooperation and T Cell-produced Interferon γ," *J Exp Med*, 178:151-161 (1993); and Cavallo F., et al., "Role of Neutrophils and $CD4^+$ T Lymphocytes in the Primary and Memory Response to Nonimmunogenic Murine Mammary Adenocarcinoma made Immunogenic by IL-2 Gene," *J Immunol*, 149(11):3627-3635 (1992); and Griffith T.S., et al., "Monocyte-mediated Tumoricidal Activity via the Tumor Necrosis Factor-related Cytokine, TRAIL," *J Exp Med*, 189(8):1343-1353 (1999); and Yoneda Y.; Yoshida R., "The Role of T

Cells in Allografted Tumor Rejection: IFN-γ Released from T Cells is Essential for Induction of Effector Macrophages in the Rejection Site," *J Immunol,* 160:6012-6017 (1998); and Noffz G. et al., "Neutrophils but not Eosinophils are Involved in Growth Suppression of IL-4-Secreting Tumors," *J Immunol,* 160:345-350 (1998); and Gerrard T.L., et al., "Human Neutrophil-Mediated Cytotoxicity to Tumor Cells," *JNCI,* 66(3):483-488 (1981); and Clark R.A.; Klebanoff S.J., "Role of the Myeloperoxidase-H_2O_2-Halide System in Concanavalin A-Induced Tumor Cell Killing by Human Neutrophils," *J Immunol,* 122(6):2605-2610 (1979); and Hafeman D.G.; Lucas Z.J., "Polymorphonuclear Leukocyte-Mediated, Antibody-Dependent, Cellular Cytotoxicity against Tumor Cells: Dependence on Oxygen and the Respiratory Burst," *J Immunol,* 123(1):55-62 (1979); and Clark R.A.; Szot S., "The Myeloperoxidase-Hydrogen Peroxide-Halide System as Effector of Neutrophil-Mediated Tumor Cell Cytotoxicity," *J Immunol,* 126(4):1295-1301 (1981); and Clark R.A.; Klebanoff S.J., "Neutrophil-mediated Tumor Cell Cytotoxicity: Role of the Peroxidase System," *J Exp Med,* 141:1442-1447 (1975); and Pericle F., et al., "CD44 is a Cytotoxic Triggering Molecule on Human Polymorphonuclear Cells," *J Immunol,* 157:4657-4663 (1996); and their contents are hereby incorporated by reference in their entirety.

A variety of mechanisms may be employed to target the innate immune system against tumors. Fundamentally this approach involves delivering, selectively to the tumor key, signal molecules that trick the immune system into regarding the tumor as a pathogenic microorgansim. A major advantage of this approach is that it is not necessary to sensitize or immunize the patient to evoke the immune

response. Potent signal molecules that can be delivered to tumors to stimulate the innate immune system include:

1.) N-formyl peptide receptor agonists

2.) Tuftsin receptor agonists

3.) Lipoxin A(4) receptor agonists

4.) Leukotriene B4 agonists

5.) 3-formyl-1-butyl-pyrophosphates receptor agonists

6.) Gal alpha(1,3)Gal. analogs

It is important that the targeting specificity of the drug be defined by the targeting ligands not by the interaction of the immune stimulator with immune effector cells. This can be accomplished by employing masked immunostimulator ligands, that are unmasked by a trigger after localization to tumor cells has occurred.

Targeted Delivery of Ligands for the Formyl Peptide Receptor

The formyl peptide receptor(s) (FPR) is a protein present on the surface of neutrophils, monocytes and macrophages that bind to n-formyl peptides with high affinity. Bacteria initiate protein translation with n-formyl methionine and the innate immune system has evolved to recognize the presence of n-formyl methionine peptides as a sign of bacterial infection. A large number of small formyl peptides such as N-formyl-Met-Leu-Phe are potent chemotactic and activating agents for leukocytes. Superoxide generation, and the release of inflammatory cytokines are potently stimulated by activation of FPR receptors.

Antibodies coupled to ligands for FPR have been explored as antitumor agents, but failed to show significant efficacy in vivo.

It is known that linkers with fluorescent groups may be attached to the carboxy terminus of N-formyl-Met-Leu-Phe without impairing affinity or biological activity, while the formyl group is critical for effective binding and activity for most, but not all analogs. For example, N-formyl-methionyl-norleucyl-leucyl-phenylalanine-phenylalanine and N-acetyl-methionyl-norleucyl-leucyl-phenylalanine-phenylalanine are both extremely potent activactors of FPR. The N unsubstituted analog is less potent, but still active at nanomolar concentrations. Certain N-terminal carbamates are also extremely potent activators of FPR.

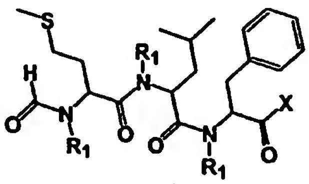
The following reference relate to this matter : Obrist R., et al., "Conjugation Behaviour of Different Monoclonal Antibodies to F-Methionyl-Leucyl-Phenylalanine," *Int J Immunopharmacol,* 8(6):629-32 (1986); and Yuli I.; Snyderman R., "Extensive Hydrolysis of N-Formyl-L-Methionyl-L-Leucyl-L-[3H] Phenylalanine by Human Polymorphonuclear Leukocytes. A Potential Mechanism for Modulation of the Chemoattractant Signal," *J Biol Chem,* 261(11):4902-8 (1986); and Bycroft B.W., et al., "Antibacterial and Immunostimulatory Properties of Chemotactic N-Formyl Peptide Conjugates of Ampicillin and Amoxicillin," *Antimicrob Agents Chemother,* 33(9):1516-21 (1989); and Obrist R., et al., "Chemotactic Monoclonal Antibody Conjugates: A Comparison of Four Different F-Met-Peptide-Conjugates," *Biochem Biophys Res Comm,* 155(3):1139-44 (1988); and Niedel J., et al., "Covalent Affinity Labeling of the Formyl Peptide Chemotactic Receptor," *J Biol Chem,* 255(15):7063-6

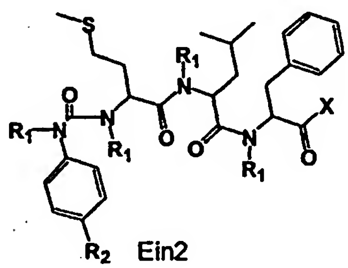
(1980); and Marasco W.A., et al., "Covalent Affinity Labeling, Detergent Solubilization, and Fluid-Phase Characterization of the Rabbit Neutrophil Formyl Peptide Chemotaxis Receptor," *Biochemistry,* 24(9):2227-36 (1985); and Niedel J., "Detergent Solubilization of the Formyl Peptide Chemotactic Receptor. Strategy Based on Covalent Affinity Labeling," *J Biol Chem,* 256(17):9295-9 (1981); and Vilven J.C., et al., "Strategies for Positioning Fluorescent Probes and Crosslinkers on Formyl Peptide Ligands," *J Recept Signal Transduct Res,* 18(2-3):187-221 (1998); and Rot A., et al., "A Series of Six Ligands for the Human Formyl Peptide Receptor: Tetrapeptides with High Chemotactic Potency and Efficacy," *Proc Natl Acad Sci USA,* 84(22):7967-71 (1987); and Mills J.S., et al., "Identification of a Ligand Binding Site in the Human Neutrophil Formyl Peptide Receptor Using a Site-Specific Fluorescent Photoaffinity Label and Mass Spectrometry," *J Biol Chem,* 273(17):10428-35 (1998); and Prossnitz E.R.; Ye R.D. "The N-Formyl Peptide Receptor: A Model for the Study of Chemoattractant Receptor Structure and Function," *Pharmacol Ther,* 74(1):73-102 (1997); and Fay S.P., et al., "Multiparameter Flow Cytometric Analysis of a pH Sensitive Formyl Peptide with Application to Receptor Structure and Processing Kinetics," *Cytometry,* 15(2):148-53 (1994); and Johansson B., et al., "N-Formyl Peptide Receptors in Human Neutrophils Display Distinct Membrane Distribution and Lateral Mobility when Labeled with Agonist and Antagonist," *J Cell Biol,* 121(6):1281-9 (1993); and Painter R.G., et al., "Photoaffinity Labeling of the N-Formyl Peptide Receptor of Human Polymorphonuclear Leukocytes," *J Cell Biochem,* 20(2):203-14 (1982); and Allen R.A., et al., "Physicochemical Properties of the N-Formyl Peptide Receptor on Human Neutrophils," *J Biol Chem,* 261(4):1854-7 (1986); and Allen R.A., et al., "Preparation and Properties

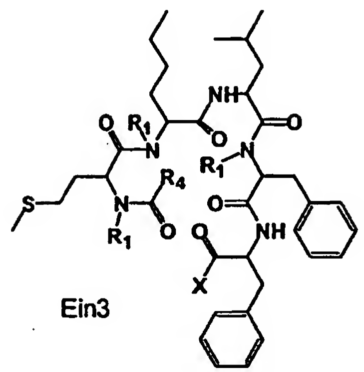
of an Improved Photoaffinity Ligand for the N-Formyl Peptide Receptor," *Biochim Biophys Acta*, 882(3):271-80 (1986); and Niedel J.E., et al., "Receptor-Mediated Internalization of Fluorescent Chemotactic Peptide by Human Neutrophils," *Science*, 205(4413):1412-4 (1979). Obrist R., et al., "Acute and Subacute Toxicity of Chemotactic Conjugates between Monoclonal Antibody and fMet-Leu-Phe in Humans: A Phase I Clinical Trial," *Cancer Immunol Immunother*, 32(6):406-8 (1991); and Obrist R.; Sandberg A.L., "Enhancement of Macrophage Invasion of Tumors by Administration of Chemotactic Factor-Antitumor Antibody Conjugates," *Cell Immunol*, 81(1):169-74 (1983); and Balazovich K.J., et al., "Tumor Necrosis Factor-Alpha and FMLP Receptors are Functionally Linked During FMLP-Stimulated Activation of Adherent Human Neutrophils," *Blood*, 88(2):690-6 (1996); and Freer R.J., et al., "Further Studies on the Structural Requirements for Synthetic Peptide Chemoattractants," *Biochemistry*, 19:2404-2410 (1980); and Gao J.L., et al., "A High Potency Nonformylated Peptide Agonist for the Phagocyte *N*-Formylpeptide Chemotactic Receptor," *J Exp Med*, 180:2191-2197 (1994); and Higgins J.D., et al., "N-Terminus Urea-Substituted Chemotactic Peptides: New Potent Agonists and Antagonists toward the Neutrophil fMLF Receptor," *J Med Chem*, 39(5):1013-1015 (1996) and their contents are hereby incorporated by reference in their entirety.

In a preferred embodiment, E is comprised of a masked ligand for FPR that is masked in a bioreversible manner. E may be configured either to tether an FPR ligand to the target or to release an FPR ligand in the microenvironment of the target.

In preferred embodiment (Ein1-Ein3), of E is comprised of the structure shown below:

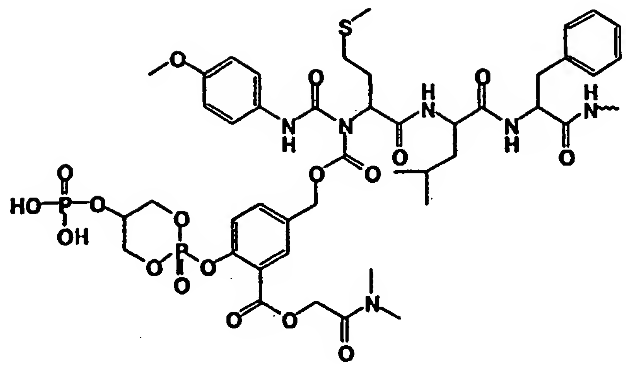

Ein1


Ein2


Ein3

wherein X is either OH or the site of linker attachment to the remainder of the target drug; and wherein R_1 is either H, or a bioreversible protecting group, or masking trigger; and R_2 is Cl, methyl, or methoxy; and R_4 is H, or methyl; and wherein either X or R1 has a site of attachment to the remainder of ET. A large number of suitable triggers are described in the trigger section of this document.

In a preferred embodiment (Ein4) , E is comprised of the following structure:



wherein the wavy line is the site of attachment to the remainder of the targeted drug. Activation of the trigger by esterase will liberate the biologically active FPR receptor activator.

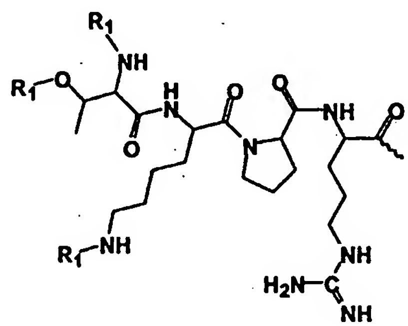
Targeted Delivery of Tuftsin Analogs

Tuftsin is the tetrapeptide threonyl-lysyl-prolyl-arginine. Tuftsin is a potent activator of granulocyte, macrophage and monocyte function. Phagocytosis, chemotaxis, hydrogen peroxide and superoxide production, and tumor necrosis factor production are all stimulated by tuftsin. NK cell activity is also markedly potentiated by tuftsin. Tuftsin exerts considerable antitumor activity in a number of animal models. A large number of tufsin analogs, which bind to the tuftsin receptor, and evoke potent activity are known. Fluorescent analogs which retain activity have been synthesized by derivatizing the C terminus of tuftsin. The following references relate to this matter: Najjar V.A.; Fridkin M., "Anitneoplastic, Immunogenic and Other Effects of the Tetrapeptide Tuftsin: A Natural Macrophage Activator," *Ann of New York Acad Sci,* 419:1-273 (1983); and Nishioka K., et al., "Antitumor Effect of Tuftsin," *Mol Cell Biochem,* 41:13-8 (1981); and Fridkin M.; Najjar V.A., "Tuftsin: Its Chemistry, Biology, and Clinical

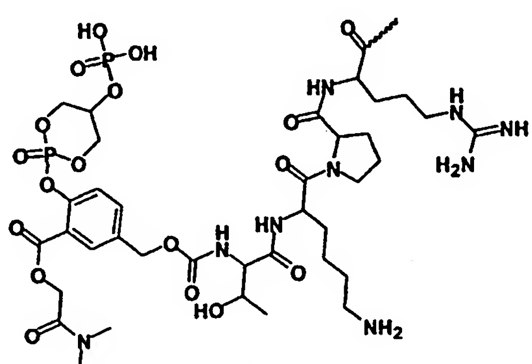
Potential," *Crit Rev Biochem Mol Biol*, 24(1):1-40 (1989); and Bar-Shavit Z., et al., "Functional Tuftsin Binding Sites on Macrophage-Like Tumor Line P388D1 and on Bone Marrow Cells Differentiated in Vitro into Mononuclear Phagocytes," *Mol Cell Biochem*, 30(3):151-5 (1980); and Verdini A.S., et al., "Immunostimulation by a Partially Modified Retro-Inverso-Tuftsin Analogue Containing Thr1 psi[NHCO](R,S)Lys2 Modification," *J Med Chem*, 34(12):3372-9 (1991); and Florentin I., et al., "In Vivo Immunopharmacological Properties of Tuftsin (Thr-Lys-Pro-Arg) and Some Analogues," *Methods Find Exp Clin Pharmacol*, 8(2):73-80 (1986); and Kraus-Berthier L., et al., "In Vivo Immunopharmacological Properties of Tuftsin and Four Analogs," *Immunopharmacology*, 25(3):261-7 (1993); and Phillips J.H., et al., "Tuftsin: a Naturally Occurring Immunopotentiating Factor. I. In Vitro Enhancement of Murine Natural Cell-Mediated Cytotoxicity," *J Immunol*, 126(3):915-21 (1981); and Siemion IZ, Kluczyk A., "Tuftsin: on the 30-Year Anniversary of Victor Najjar's Discovery," *Peptides*, 20(5):645-74 (1999); and Gottlieb P., et al., "Receptor-Mediated Endocytosis of Tuftsin by Macrophage Cells," *Biochem Biophys Res Commun*, 119(1):203-11 (1984); and Dagan S., et al., "Tuftsin Analogues: Synthesis, Structure-Function Relationships, and Implications for Specificity of Tuftsin's Bioactivity," *J Med Chem*, 29(10):1961-8 (1986); and Bump N.J., et al., "The characteristics of Purified HL60 Tuftsin Receptors," *Mol Cell Biochem*, 92(1):77-84 (1990); and Cillari E., et al., "The Macrophage-Activating Tetrapeptide Tuftsin Induces Nitric Oxide Synthesis and Stimulates Murine Macrophages to Kill Leishmania Parasites In Vitro," *Infect Immun*, 62(6):2649-52 (1994); and Tzehoval E., et al., "Tuftsin (an Ig-associated Tetrapeptide) Triggers the Immunogenic Function of Macrophages: Implications

for Activation of Programmed Cells," *Proc Natl Acad Sci USA*, 75(7):3400-4 (1978); and Bar-Shavit Z., et al., "Tuftsin-Macrophage Interaction: Specific Binding and Augmentation of Phagocytosis," *J Cell Physiol*, 100(1):55-62 (1979); and the contents are hereby incorporated by reference in their entirety.

In a preferred embodiment, E is comprised of a masked tufsin receptor activator that is masked in a bioreversible fashion. E may be configured either to a tuftsin receptor agonist to the target or to release it in the microenvironment of the target. In a preferred embodiment (Ein5), E has the structure:



wherein the wavy line is the site of attachment of the remainder of the targeted drug and R_1 is H, or a masking trigger which when activated generates the biologically active tuftsin agonist. In a preferred embodiment (Ein6), E has the following structure:



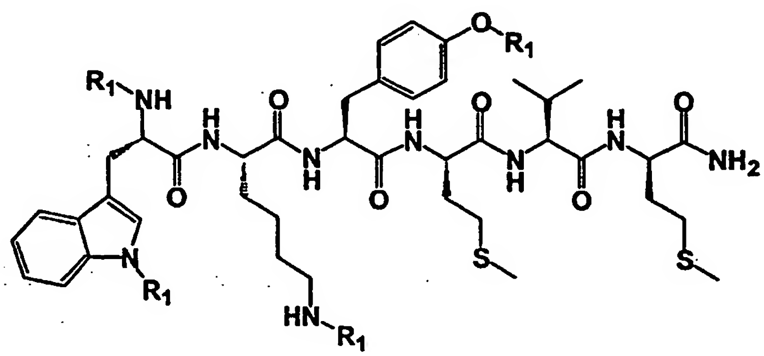
Activation of the clock-like time delayed masking trigger by esterase will liberate the biologically active Tuftsin receptor agonist.

Targeted Delivery of Lipoxin A4 Receptor Activators

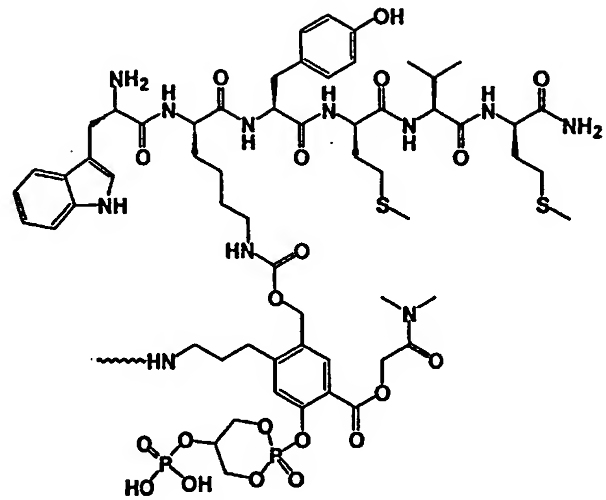
The oligopeptide Trp-Lys-Tyr-Met-Val-D-Met-NH2 is an extremely potent chemotactic agent, which activates neutrophils and monocytes to produce hydrogen peroxide and superoxide, and release inflammatory cytokines. This activity is mediated by binding to the lipoxin A4 receptor at picomolar concentrations. At nanomolar concentration the FPR is also activated. The following references relate to this matter: Seo J.K., et al., "A Peptide with Unique Receptor Specificity: Stimulation of Phosphoinositide Hydrolysis and Induction of Superoxide Generation in Human Neutrophils," *J Immunol,* 158(4):1895-901 (1997); and Bae Y.S., et al., "Trp-Lys-Tyr-Met-Val-D-Met is a Chemoattractant for Human Phagocytic Cells," *J Leukoc Biol,* 66(6):915-22 (1999); and Bae Y.S., et al., "Trp-Lys-Tyr-Met-Val-D-Met Stimulates Superoxide Generation and Killing of Staphylococcus Aureus via Phospholipase D Activation in Human Monocytes," *J Leukoc Biol,* 65(2):241-8 (1999); and Dahlgren C., et al., "The Synthetic Chemoattractant Trp-Lys-Tyr-Met-Val-Dmet Activates Neutrophils Preferentially through the Lipoxin A(4) Receptor," *Blood,* 95(5):1810-8 (2000); and Le Y., et al., "Utilization of Two Seven-Transmembrane, G Protein-Coupled Receptors, Formyl Peptide Receptor-Like 1 and Formyl Peptide Receptor, by the Synthetic Hexapeptide WKYMVm for Human Phagocyte Activation." *J Immunol,* 163(12):6777-84 (1999); and Seo J.K., et al., "Distribution of the Receptor for a Novel Peptide Stimulating Phosphoinositide Hydrolysis in Human

Leukocytes," *Clin Biochem,* 31(3):137-41 (1998); and the contents are hereby incorporated by reference in their entirety..

In a preferred embodiment E is an actiivator of the lipoxin A4 receptor. In a preferred embodiment (Ein7),, E is comprised of the following structure structure:



wherein the Met is the D isomer and R_1 is H or a trigger which when activated generates the biologically active lipoxin A4 receptor agonist; and wherein R_1 bears a site of attachment to the remainder of the targeted drug. In a preferred embodiment (Ein8), E is comprised of the following structure:



wherein the wavy line is the site of linker attachment to the remainder of ET. Esterase can activate the clock-like time delayed masking trigger which will free the lipoxin A4 agonist from the targeted drug complex.

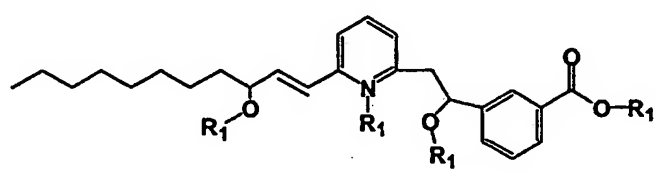
Targeted Leukotriene B4 Agonists

Leukotriene B is a potent inflammatory mediator with chemotactic and neutrophil/monocyte activating properties. Neutrophil degranulation, superoxide production and vascular permeability are all markedky increased by leukotriene B4. Leukotriene B production is dramatically increased by phospholipase A2. Phospholipase A2 activators have been reported to induce massive inflammation in gliomas and produce tumor regression in animals. Because of its importance in inflammation, extensive research has focused on the development of antagonists for leukotriene B4. In the course of these studies, a large number of extremely potent leukotriene B4 agonists have been discovered. The following references relate to this matter: Soyombo O., et al., "Structure/Activity Relationship of Leukotriene B4 and its Structural Analogues in Chemotactic, Lysosomal-Enzyme Release and Receptor-Binding Assays," *Eur J*

Biochem, 218(1):59-66 (1993); and Leblanc Y., et al., "Analogs of Leukotriene B4: Effects of Modification of the Hydroxyl Groups on Leukocyte Aggregation and Binding to Leukocyte Leukotriene B4 Receptors," *Prostaglandins,* 33(5):617-25 (1987); and Gapinski D.M., et al., "Benzophenone Dicarboxylic Acid Antagonists of Leukotriene B4. 2. Structure-Activity Relationships of the Lipophilic Side Chain," *J Med Chem,* 33(10):2807-13 (1990); and Bomalaski J.S.; Mong S., "Binding of Leukotriene B4 and its Analogs to Human Polymorphonuclear Leukocyte Membrane Receptors," *Prostaglandins,* 33(6):855-67 (1987); and Jackson W.T., et al., "Design, Synthesis, and Pharmacological Evaluation of Potent Xanthone Dicarboxylic Acid Leukotriene B4 Receptor Antagonists," *J Med Chem,* 36(12):1726-34 (1993); and Hoover R.L., et al., "Leukotriene B4 Action on Endothelium Mediates Augmented Neutrophil/Endothelial Adhesion," *Proc Natl Acad Sci USA,* 81(7):2191-3 (1984); and Palmblad J., et al., "Leukotriene B4 Triggers Highly Characteristic and Specific Functional Responses in Neutrophils: Studies of Stimulus Specific Mechanisms," *Biochim Biophys Acta,* 871(1):92-102 (1988); and Crooks S.W.; Stockley R.A., "Leukotriene B4," *Int J Biochem Cell Biol,* 30(2):173-8 (1998); and Lam B.K., et al., "Phospholipase A2 as Leukotriene B4 Secretagogue for Human Polymorphonuclear Leukocytes," *Adv Exp Med Biol,* 275:183-91 (1990); and Goddard D.H., et al., "Phospholipase A2-Mediated Inflammation Induces Regression of Malignant Gliomas," *Cancer Lett,* 102(1-2):1-6 (1996); and Daines R.A., et al., "Trisubstituted Pyridine Leukotriene B4 Receptor Antagonists: Synthesis and Structure-Activity Relationships," *J Med Chem,* 36(22):3321-32 (1993); and Poudrel J.M., et al., "Synthesis and Structure-Activity Relationships of New 1,3-Disubstituted Cyclohexanes as Structurally Rigid Leukotriene B_4

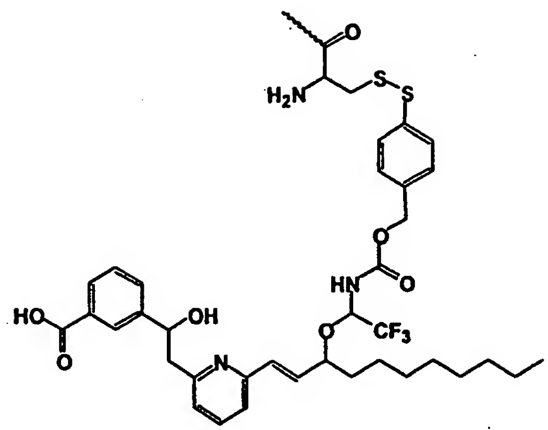
Receptor Antagonists," *J Med Chem*, 42(26):5289-5310 (1999); and Kingsbury W.D., et al., "Synthesis of Structural Analogs of Leukotriene B4 and their Receptor Binding Activity," *J Med Chem*, 36(22):3308-20 (1993); and Konno M., et al., "Synthesis of Structural Analogues of Leukotriene B4 and their Receptor Binding Activity," *Bioorg Med Chem*, 5(8):1621-47 (1997); and the contents are hereby incorporated by reference in their entirety.

In a preferred embodiment, E is comprised of a leukotriene B4 agonist. In a preferred embodiment (Ein9), E is comprised of the following structure:



wherein R_1 is H, or the site of attachment to the remainder of the targeted drug.

In a preferred embodiment (Ein10), E has the following structure:



wherein the wavy line is the site of attachment to the remainder of the drug complex. Cleavage of the disulfide bond will free the leukotriene B4 agonist.

Targeted Delivery of γ/δ T Cell Activators

γ/δ T cells are a class of lymphocytes, which recognize antigens in a manner analogous to antibodies in the absence of MHC restriction. γ/δ T cells have been implicated in immunity to tuberculosis, malaria, listeria, and herpes simplex virus. Contact hypersensitivity, autoimmunity, graft versus host disease, and tumor rejection have all been associated with γ/δ T cells. γ/δ T cells produce target damage by perforin mediated cytotoxicity, and the release of a variety of cytokines such as interferon gamma, macrophage inflammatory protein, lymphotactin, RANTES, and tumour necrosis factor alpha.

A high percentage of human γ/δ T cells are activated by phosphoantigens derived from mycobacterium such as prenyl pyrophosphate analogs. 3-Formyl – 1-butyl-pyrophosphate and related derivatives are extremely potent activators of γ/δ T cells. The following references relate to this matter:

Belmant C, et al., "3-Formyl-1-butyl Pyrophosphate a Novel Mycobacterial Metabolite-Activating Human Gammadelta T Cells," *J Biol Chem*, 274(45):32079-84 (1999); and Huber H., et al., "Activation of Murine Epidermal TCR-Gamma Delta+ T Cells by Keratinocytes Treated with Contact Sensitizers," *J Immunol*, 155(6):2888-94 (1995); and Groh V., et al., "Broad Tumor-Associated Expression and Receognition by Tumor-Derived γδ T Cells of MICA and MICB," *PNAS*, 96(12):6879-6884 (1999); and Sciammas R., et al., "T Cell Receptor-Gamma/Delta Cells Protect Mice from Herpes Simplex Virus Type 1-Induced Lethal Encephalitis," *J Exp Med*, 185(11):1969-75 (1997); and Bukowski J.F., et al., "Crucial Role of TCR Gamma Chain Junctional Region in Prenyl Pyrophosphate Antigen Recognition by Gamma Delta T Cells," *J Immunol*, 161(1):286-93 (1998); and Zocchi M.R., et al., "Selective Lysis of the Autologous Tumor by Delta TCS1+ Gamma/Delta+ Tumor-Infiltrating Lymphocytes from Human Lung Carcinomas," *Eur J Immunol*, 20(12):2685-9 (1990); and Morita C.T., et al., "Direct Presentation of Nonpeptide Prenyl Pyrophosphate Antigens to Human Gamma Delta T Cells," *Immunity*, 3(4):495-507 (1995); and Elloso M.M., et al., "The Effects of Interleukin-15 on Human Gammadelta T Cell Responses to Plasmodium Falciparum in Vitro," *Immunol Lett*, 64(2-3):125-32 (1998); and Ferrarini M., et al., "Killing of Laminin Receptor-Positive Human Lung Cancers by Tumor Infiltrating Lymphocytes Bearing Gammadelta(+) T-Cell Receptors," *J Natl Cancer Inst*, 88(7):436-41 (1996); and Maeurer M.J., et al., "Human Intestinal Vdelta1+ Lymphocytes Recognize Tumor Cells of Epithelial Origin," *J Exp Med*, 183(4):1681-96 (1996); and Yu S., et al., "Expansion and Immunological Study of Human Tumor Infiltrating Gamma/Delta T Lymphocytes In Vitro," *Int Arch Allergy Immunol*, 119(1):31-7 (1999); and

Weintraub B.C., et al., "Gamma Delta T Cells can Recognize Nonclassical MHC in the Absence of Conventional Antigenic Peptides," *J Immunol,* 153(7):3051-8 (1994); and Kabelitz D, et al. "Gamma Delta T Cells, their T Cell Receptor Usage and Role in Human Diseases," *Springer Semin Immunopathol, 21(1):55-75 (1999); and* Cipriani B., et al., "Activation of C-C Beta-Chemokines in Human Peripheral Blood Gammadelta T Cells by Isopentenyl Pyrophosphate and Regulation by Cytokines," *Blood,* 95(1):39-47 (2000); and Bukowski J.F., et al., "Human Gamma Delta T Cells Recognize Alkylamines Derived from Microbes, Edible Plants, and Tea: Implications for Innate Immunity," *Immunity,* 11(1):57-65 (1999); and Laad A.D, et al., "Human Gamma Delta T Cells Recognize Heat Shock Protein-60 on Oral Tumor Cells," *Int J Cancer,* 80(5):709-14 (1999); and Burk M.R., et al., "Human V Gamma 9-V Delta 2 Cells are Stimulated in a Cross-Reactive Fashion by a Variety of Phosphorylated Metabolites," *Eur J Immunol,* 25(7):2052-8 (1995); and Garcia V.E., et al., "IL-15 Enhances the Response of Human Gamma Delta T Cells to Nonpeptide [Correction of Nonpetide] Microbial Antigens," *J Immunol,* 160(9):4322-9 (1998); and Chandler P., et al., "Immune Responsiveness in Mutant Mice Lacking T-Cell Receptor Alpha Beta+ Cells," *Immunology,* 85(4):531-7 (1995); and Dechanet J., et al., "Implication of Gammadelta T Cells in the Human Immune Response to Cytomegalovirus," *J Clin Invest,* 103:10):1437-49 (1999); and Wei Y., et al., "Induction of Autologous Tumor Killing by Heat Treatment of Fresh Human Tumor Cells: Involvement of Gamma Delta T Cells and Heat Shock Protein 70," *Cancer Res,* 56(5):1104-10 (1996); and Blazar B.R., et al., "Lethal Murine Graft-Versus-Host Disease Induced by Donor Gamma/Delta Expressing T Cells with Specificity for Host Nonclassical Major Histocompatibility Complex Class Ib

Antigens," *Blood,* 87(2):827-37 (1996); and Bialasiewicz A.A., et al., "Alpha/Beta- and Gamma/Delta TCR(+) Lymphocyte Infiltration in Necrotising Choroidal Melanomas," *Br J Ophthalmol,* 83(9):1069-73 (1999); and Gan Y.H.; Malkovsky M., "Mechanisms of Simian Gamma Delta T Cell Cytotoxicity against Tumor and Immunodeficiency Virus-Infected Cells," *Immunol Lett,* 49(3):191-6 (1996) Manfredi A.A., et al., "Mycobacterium Tuberculosis Exploits the CD95/CD95 Ligand System of Gammadelta T Cells to Cause Apoptosis," *Eur J Immunol,* 28(6):1798-806 (1998); and Tanaka Y., et al., "Natural and Synthetic Non-Peptide Antigens Recognized by Human Gamma Delta T Cells," *Nature,* 375(6527):155-8 (1995); and Tanaka Y., et al., "Nonpeptide Ligands for Human Gamma Delta T Cells," *Proc Natl Acad Sci USA,* 91(17):8175-9 (1994); and Poccia F., et al., "Phosphoantigen-Reactive Vgamma9Vdelta2 T Lymphocytes Suppress in Vitro Human Immunodeficiency Virus Type 1 Replication by Cell-Released Antiviral Factors including CC Chemokines," *J Infect Dis,* 180(3):858-61 (1999); and De Libero G., et al., "Selection by Two Powerful Antigens may Account for the Presence of the Major Population of Human Peripheral Gamma/Delta T Cells," *J Exp Med,* 173(6):1311-22 (1991); and Boullier S., et al., "Regulation by Cytokines (IL-12, IL-15, IL-4 and IL-10) of the Vgamma9Vdelta2 T Cell Response to Mycobacterial Phosphoantigens in Responder and Anergic HIV-Infected Persons," *Eur J Immunol,* 29(1):90-9 (1999); and Ferrini S., et al., "Retargeting of T-Cell-Receptor Gamma/Delta+ Lymphocytes against Tumor Cells by Bispecific Monoclonal Antibodies. Induction of Cytolytic Activity and Lymphokine Production," *Int J Cancer Suppl,* 4:53-5 (1989); and Salerno A., et al., "Role of Gamma Delta T Lymphocytes in Immune Response in Humans and Mice," *Crit Rev Immunol,* 18(4):327-57

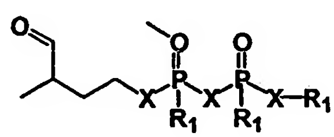
(1998); and Choudhary A., et al., "Selective Lysis of Autologous Tumor Cells by Recurrent Gamma Delta Tumor-Infiltrating Lymphocytes from Renal Carcinoma," *J Immunol*, 154(8):3932-40 (1995); and Catalfamo M., et al., "Self-Reactive Cytotoxic Gamma Delta T Lymphocytes in Graves' Disease Specifically Recognize Thyroid Epithelial Cells," *J Immunol*, 156(2):804-11 (1996); and Constant P., et al., "Stimulation of Human Gamma Delta T Cells by Nonpeptidic Mycobacterial Ligands," *Science*, 264(5156):267-70 (1994); and Li H., et al., "Structure of the Vdelta Domain of a Human Gammadelta T-Cell Antigen Receptor," *Nature*, 391(6666):502-6 (1998); and Zhao X., et al., "Accumulation of Gamma/Delta T Cells in Human Dysgerminoma and Seminoma: Roles in Autologous Tumor Killing and Granuloma Formation," *Immunol Invest*, 24(4):607-18 (1995); and Cipriani B., et al., "Activation of C-C-β-Chemokines in Human Peripheral Blood γδ T Cells by Isopentenyl Pyrophosphate and Regulation by Cytokines," *Blood*, 95(1):39-47 (2000); and Bialasiewicz A.A., et al., "Alpha/Beta- and Gamma/Delta TCR(+) Lymphocyte Infiltration in Necrotising Choroidal Melanomas," *Br J Ophthalmol*, 83(9):1069-73 (1999); and Yin Z., et al., "Dominance of IL-12 over IL-4 in Gamma Delta T Cell Differentiation Leads to Default Production of IFN-Gamma: Failure to Down-Regulate IL-12 Receptor Beta 2-Chain Expression," *J Immunol*, 164(6):3056-64 (2000); and Thomas M.L., et al., "Gammadelta T Cells Lyse Autologous and Allogenic Oesophageal Tumours: Involvement of Heat-Shock Proteins in the Tumour Cell Lysis," *Cancer Immunol Immunother*, 48(11):653-9 (2000); and Fujimiya Y., et al., "In Vitro Interleukin 12 Activation of Peripheral Blood CD3(+)CD56(+) and CD3(+)CD56(-) Gammadelta T Cells from Glioblastoma Patients," *Clin Cancer Res*, 3(4):633-43 (1997); and Yamaguchi T., et al.,

"Interleukin-15 Effectively Potentiates the in Vitro Tumor-Specific Activity and Proliferation of Peripheral Blood Gammadelta T Cells Isolated from Glioblastoma," *Cancer Immunol Immunother,* 47(2):97-103 (1998); and Poccia F., et al., "Phosphoantigen-Reactive Vgamma9Vdelta2 T Lymphocytes Suppress in Vitro Human Immunodeficiency Virus Type 1 Replication by Cell-Released Antiviral Factors Including CC Chemokines," *J Infect Dis,* 180(3):858-61 (1999); and Wesch D., et al., "Comparative Analysis of Alpha Beta and Gamma Delta T Cell Activation by Mycobacterium Tuberculosis and Isopentenyl Pyrophosphate," *Eur J Immunol,* 27(4):952-6 (1997); and the contents are hereby incorporated by reference in their entirety.

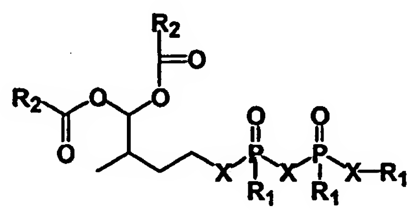
In a preferred embodiment, E is an activator of γ/δ T cells which is masked in a bioreversible manner.

In preferred embodiments (Ein11 and Ein12), E is comprised of the following structures:

Ein11



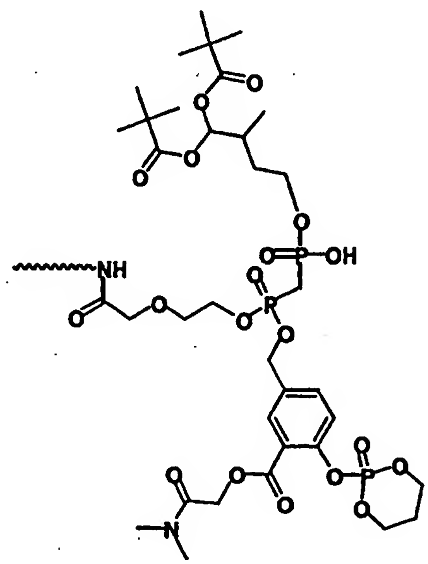
Ein12



wherein X is O, or CH_2, and R_1 is OH, a bioreversible masking group, or a site of attachment to the remainder of the targeted drug, and R_2 is a lower alkyl group, or a phenyl group, or other group such that the resulting ester is cleaved by

esterase; and wherein R_2 may also bear a site of attachment to the remainder of ET.

In a preferred embodiment (Ein13), E is comprised of the following structure:



In this embodiment, the active formyl analog is generated following cleavage of the pivalate by esterase and following triggering of the clock-like time delayed trigger by esterase.

In a preferred embodiment, E is comprised of two masked activators of γ/δ T cells, which are masked in a bioreversible manner, connected by a linker, which is connencted to the remainder of the targeted drug; wherein the linker is selected so as to allow bivalent binding to the γ/δ T cell of the unmasked formyl pyrophosphate ligands.

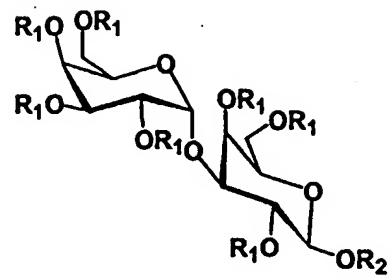
Targeted Delivery of alpha-Galactosyl Epitopes

Humans naturally produce high titre antibodies to terminal α-galactosyl-(1, 3)-β–galactosyl structures. These antibodies mediate the hyperacute rejection of xenographs. In addition, NK cells recognize terminal α-galactosyl-(1, 3)Galβ structures as targets. Gene transfer of alpha(1,3)galactosyltransferase into tumor cells has been been explored as a means of inducing α-galactosyl directed immune responses against tumors. The following references relate to this matter: Fang J., et al., "A Unique Chemoenzymatic Synthesis of α-Galactosyl Epitope Derivatives Containing Free Amino Groups: Efficient Separation and Further Manipulation," *J Org Chem,* 64(11):4089-4094 (1999); and Janczuk A., et al., "Alpha-Gal Oligosaccharides: Chemistry and Potential Biomedical Application," *Curr Med Chem,* 6(2):155-64 (1999); and Galili U., "Abnormal Expression of Alpha-Galactosyl Epitopes in Man. A Trigger for Autoimmune Processes?" *Lancet,* 2(8659):358-61 (1989); and Jager U., et al., "Induction of Complement Attack on Human Cells by Gal(Alpha1,3)Gal Xenoantigen Expression as a Gene Therapy Approach to Cancer," *Gene Ther,* 6(6):1073-83 (1999); and Artrip J.H., et al., "Target Cell Susceptibility to Lysis by Human Natural Killer Cells is Augmented by α(1,3)-Galactosyltransferase and Reduced by α(1,2)-Fucosyltransferase-," *J Biol Chem,* 274(16):10717-10722 (1999); and Vaughan H.A., et al., "Gal alpha(1,3)Gal is the Major Xenoepitope Expressed on Pig Endothelial Cells Recognized by Naturally Occurring Cytotoxic Human Antibodies," *Transplantation,* 58(8):879-82 (1994); and Inverardi L., et al., "Human Natural Killer Lymphocytes Directly Recognize Evolutionarily Conserved Oligosaccharide Ligands Expressed by Xenogeneic Tissues," *Transplantation,* 63(9):1318-30 (1997); and Ni Y., et al., "Specificity

and Prevalence of Natural Bovine Anti-Alpha Galactosyl (Gal^{α}1-6Glc or Gal^{α}-16Gal) Antibodies," *Clin Diagnostic Lab Immunol,* 7(3):490-496 (2000); and LaTemple D.C. et al., "Synthesis of Alpha-Galactosyl Epitopes by Recombinant Alpha1,3galactosyl Transferase for Opsonization of Human Tumor Cell Vaccines by Anti-Galactose," *Cancer Res,* 56(13):3069-74 (1996); and Galili U., et al., "Human Natural Anti-Alpha-Galactosyl IgG. II. The Specific Recognition of Alpha (1, 3)-Linked Galactose Residues," *J Exp Med,* 162:573-582 (1985); and Link C.J. Jr., et al., "Eliciting Hyperacute Xenograft Response to Treat Human Cancer: Alpha(1,3) Galactosyltransferase Gene Therapy," *Anti-cancer Res,* 18(4A):2301-8 (1998); and the contents are hereby incorporated by reference in their entirety.

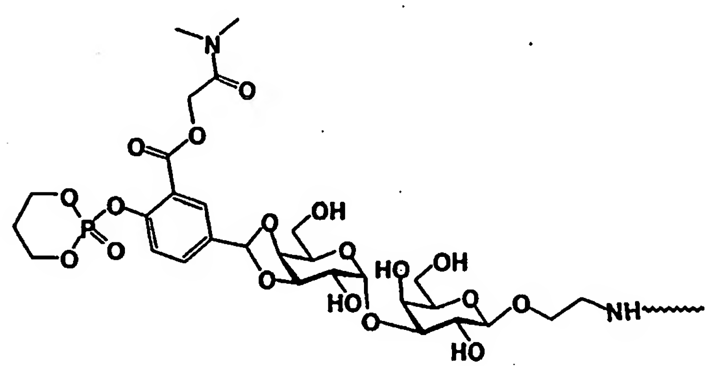
The intense innate immunity that pre-exists in humans to these epitopes can be targeted against tumors by selectively delivering masked terminal α-galactosyl-(1, 3)Galβ structures to tumors. In a preferred embodiment, E is comprised of one or more masked terminal α-galactosyl-(1-3)Galβ structures. Addition of a bioreversible masking group to one or more of the hydroxy groups on the disaccharide will alter the conformation and preclude antibody binding. Unmasking following tumor localization will expose the epitope and trigger an intense antitumor response.

In a preferred embodiment (Ein14), E is comprised of the following structure:



wherein R_1 is OH or a bioreversible masking group which when unmasked exposes the hydroxy group, and R_2 (which may bear additional sugar residues) is the site of linker attachment to the remainder of the drug. R_1 can be an ester, phosphate, acetal, carbonate, or any group which can generate the free hydroxy group by spontaneous or biochemical mechanisms.

In a preferred embodiment (Ein15), E has the following structure:



wherein the wavy line is the site of attachment to the remainder of the drug complex. Activation of the clock-like time delayed trigger by esterase will trigger acetal hydrolysis by stabilizing the carbocation formation at the benzylic carbon and unmask the antigen.

Multifactorial Targeting with Sets of Monofactorial Drugs and Multiple Set-MultiFactorial Targeting

Although a single property or characteristic is not unique to malignant cells the pattern of expression of multiple properties may provide almost absolute tumor specificity. Multifunctional drug delivery vehicles provide one means to accomplish multifactorial targeting. This section describes a complementary technology that may be used to achieve highly selective multifactorial targeting by using multiple independently targeted drugs to deliver multiple effector agents, wherein the effector agents individually have low toxicity, but jointly are highly toxic. This technology may be employed with monofactorially targeted drugs or with multifunctional drug delivery vehicles. When applied to multifunctional drug delivery vehicles, this technology will restrict the targeting domain of toxicity to cells that jointly express both sets of properties targeted by each multifunctional drug delivery vehicle.

This invention relates to the compositions, targets and methods of use of independent sets of targeted drugs; wherein the targeted drugs individually have low toxicity, but the combination of one or more of the drugs is potently toxic for cells that are jointly targeted. Any combination of effector agents that display potent synergystic toxicity, and which individually are of much lower toxicity may be employed. In this technology, multifatorial targeting occurs at the effector level.

Synergystic combinations of conventional drugs with enhanced toxicity are well known in cancer chemotherapy. Grosveld disclosed a means of targeting genes and regulatory elements, which functionally cooperate *inside* the cell using different independent targeting ligands. Combinations of targeted immunotoxins that exert synergystic toxicity are also known. The following references relate to this matter: 5,849,718 12/15/98 Grosveld, "Targeting Complexes and Use Thereof".; and Crews J.R., et al., "A Combination of Two Immunotoxins Exerts Synergistic Cytotoxic Activity Against Human Breast-Cancer Cell Lines," *Int J Cancer,* 51:772-779 (1992) and the contents are hereby incorporated by reference in their entirety.

The combination of antimetabolites, which interfere with the denovo synthesis of a factor essential for cell growth and survival with inhibitors that block the salvage pathways related to the factor, may display striking synergystic toxicity. Examples include:

1.) Inhibitors of purine and pyrimidine synthesis in combination with nucleoside transport inhibitors; and

2.) Inhibitors of polyamine synthesis and polyamine transport inhibitors.

One embodiment of the present invention comprises a set of n targeted drugs referred to as "E1-T1" ..."En-Tn" wherein E1...En comprise effector groups which in combination exert synergystic toxicity, and T1...Tn comprise different targeting ligands. (A large number of targeting ligands and tumor-selective targeting ligands have been detailed in other sections and apply to this embodiment of the present invention.) The present invention also relates to the method in which this set of drugs is administered, in combination, alone or in

conjunction with non-targeted drugs that further potentiate selective toxicity for the treatment of neoplastic disease.

A preferred embodiment (referred to as embodiment "PET1") of the present invention comprises the set of n different drugs referred to as "E1T1" ..."EnTn" wherein EnTn is a compound comprised of one or more effector agents referred to as "En.v" having pharmacological activity designated as "PA" and wherein Tn comprises:

a) A group comprised of at least one structure referred to as a "targeting ligand" which selectively binds to a target receptor on the surface of the target cell or in the microenvironment of the target cell; and

And wherein the different drugs E1T1...EnTn bind to different types of target receptors; and wherein the different effector groups E1...En can evoke pharmacological activitites that are synergistic; wherein synergistic means that the pharmacological activity produced by the effector groups E1...En is greater than the additive pharmacological activity of the individual effector groups acting independently ;

and wherein n is at least two; and n=1,2,3,4,5,6,7,8,9,10,11,12,13,14 or about 15; preferably N is two or three;

and wherein v is 1,2,3, or about 4, and preferably v is 1 or 2;

and wherein the drugs E1T1...EnTn are combined; wherein combined means that the drugs are present in the same solution (or liquid phase) or volume of space before being given to a patient or become so in a patient.

A preferred embodiment (embodiment PET2) of the above comprises the set of drugs E1-T1 ...En-Tn wherein En-Tn is comprised of the following groups:

a) N1 targeting ligands, which can differ;

b) N2 masked intracellular transport ligands which can differ;

c) N3 triggers, which can differ, designated "detoxification triggers" wherein activation of the trigger decreases the pharmacological activity PA;

d) N4 effector agents which can differ;

e) N5 triggers which can differ, wherein activation of the trigger increases the pharmacological activity PA;

f) N6 intracellular trapping ligands or masked intracellular trapping ligands, which can differ;

and wherein:

N1 = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or about 10; N2 = 0, 1, 2, 3, 4, or 5;

N3 = 0, 1, 2, 3, 4, 5, or about 5; N4 = 1, 2, 3, 4, 5, or about 5;

N5 = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or about 10; and

N6 = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or about 10;

and wherein the components are covalently coupled directly or by one or more linkers, and wherein the connectivity between groups can vary provided that the functionality of the different components remains intact and wherein the function of ligands is to bind to their respective receptors; the function of the triggers is to be activated and modulate drug activity, and the function of the effector agent is to evoke the pharmacological activity PA;

and wherein the linker lengths can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, ...300 bond lengths or about 300 bond lengths; wherein the (...) are meant to represent the continuation of the sequence of numbers up to 300.

In a preferred embodiment (embodiment PET3) of the above:

N1 =1, 2, 3, or 4;	N2 =0, 1, or 2;
N3 =0, 1, or 2;	N4 = 1, 2, or 3;
N5 =0, 1, 2, or 3;	N6 =1, 2, or 3;

Additional preferred embodiments (referred to as embodiment PET4.x wherein is X= the number of the line below for X=1,2,3...383) of EnTn are listed on each line below wherein:

1) N1=1, N2=0, N3=1, N4=1, N5=0, and N6=0
2) N1=1, N2=0, N3=0, N4=2, N5=0, and N6=0
3) N1=1, N2=0, N3=0, N4=3, N5=0, and N6=0
4) N1=1, N2=0, N3=0, N4=1, N5=1, and N6=0
5) N1=1, N2=0, N3=0, N4=1, N5=2, and N6=0
6) N1=1, N2=0, N3=0, N4=1, N5=3, and N6=0
7) N1=1, N2=0, N3=0, N4=1, N5=0, and N6=1
8) N1=1, N2=0, N3=1, N4=2, N5=0, and N6=0
9) N1=1, N2=0, N3=1, N4=3, N5=0, and N6=0
10) N1=1, N2=0, N3=1, N4=1, N5=1, and N6=0
11) N1=1, N2=0, N3=1, N4=1, N5=2, and N6=0
12) N1=1, N2=0, N3=1, N4=1, N5=3, and N6=0
13) N1=1, N2=0, N3=1, N4=1, N5=0, and N6=1

14) N1=1, N2=0, N3=1, N4=2, N5=1, and N6=0

15) N1=1, N2=0, N3=1, N4=2, N5=1, and N6=1

16) N1=1, N2=0, N3=1, N4=2, N5=2, and N6=0

17) N1=1, N2=0, N3=1, N4=2, N5=2, and N6=1

18) N1=1, N2=0, N3=1, N4=2, N5=3, and N6=0

19) N1=1, N2=0, N3=1, N4=2, N5=3, and N6=1

20) N1=1, N2=0, N3=1, N4=2, N5=0, and N6=1

21) N1=1, N2=0, N3=1, N4=3, N5=1, and N6=0

22) N1=1, N2=0, N3=1, N4=3, N5=1, and N6=1

23) N1=1, N2=0, N3=1, N4=3, N5=2, and N6=0

24) N1=1, N2=0, N3=1, N4=3, N5=2, and N6=1

25) N1=1, N2=0, N3=1, N4=3, N5=3, and N6=0

26) N1=1, N2=0, N3=1, N4=3, N5=3, and N6=1

27) N1=1, N2=0, N3=1, N4=3, N5=0, and N6=1

28) N1=1, N2=0, N3=1, N4=1, N5=1, and N6=1

29) N1=1, N2=0, N3=1, N4=1, N5=2, and N6=1

30) N1=1, N2=0, N3=1, N4=1, N5=3, and N6=1

31) N1=1, N2=1, N3=0, N4=1, N5=0, and N6=0

32) N1=1, N2=1, N3=0, N4=2, N5=0, and N6=0

33) N1=1, N2=1, N3=0, N4=3, N5=0, and N6=0

34) N1=1, N2=1, N3=0, N4=1, N5=1, and N6=0

35) N1=1, N2=1, N3=0, N4=1, N5=2, and N6=0

36) N1=1, N2=1, N3=0, N4=1, N5=3, and N6=0

37) N1=1, N2=1, N3=0, N4=1, N5=0, and N6=1

38) N1=1, N2=1, N3=0, N4=2, N5=1, and N6=0

39) N1=1, N2=1, N3=0, N4=2, N5=1, and N6=1

40) N1=1, N2=1, N3=0, N4=2, N5=2, and N6=0

41) N1=1, N2=1, N3=0, N4=2, N5=2, and N6=1

42) N1=1, N2=1, N3=0, N4=2, N5=3, and N6=0

43) N1=1, N2=1, N3=0, N4=2, N5=3, and N6=1

44) N1=1, N2=1, N3=0, N4=2, N5=0, and N6=1

45) N1=1, N2=1, N3=0, N4=3, N5=1, and N6=0

46) N1=1, N2=1, N3=0, N4=3, N5=1, and N6=1

47) N1=1, N2=1, N3=0, N4=3, N5=2, and N6=0

48) N1=1, N2=1, N3=0, N4=3, N5=2, and N6=1

49) N1=1, N2=1, N3=0, N4=3, N5=3, and N6=0

50) N1=1, N2=1, N3=0, N4=3, N5=3, and N6=1

51) N1=1, N2=1, N3=0, N4=3, N5=0, and N6=1

52) N1=1, N2=1, N3=0, N4=1, N5=1, and N6=1

53) N1=1, N2=1, N3=0, N4=1, N5=2, and N6=1

54) N1=1, N2=1, N3=0, N4=1, N5=3, and N6=1

55) N1=1, N2=1, N3=1, N4=1, N5=0, and N6=0

56) N1=1, N2=1, N3=1, N4=2, N5=0, and N6=0

57) N1=1, N2=1, N3=1, N4=3, N5=0, and N6=0

58) N1=1, N2=1, N3=1, N4=1, N5=1, and N6=0

59) N1=1, N2=1, N3=1, N4=1, N5=2, and N6=0

60) N1=1, N2=1, N3=1, N4=1, N5=3, and N6=0

61) N1=1, N2=1, N3=1, N4=1, N5=0, and N6=1

62) N1=1, N2=1, N3=1, N4=2, N5=1, and N6=0

63) N1=1, N2=1, N3=1, N4=2, N5=1, and N6=1

64) N1=1, N2=1, N3=1, N4=2, N5=2, and N6=0

65) N1=1, N2=1, N3=1, N4=2, N5=2, and N6=1

66) N1=1, N2=1, N3=1, N4=2, N5=3, and N6=0

67) N1=1, N2=1, N3=1, N4=2, N5=3, and N6=1

68) N1=1, N2=1, N3=1, N4=2, N5=0, and N6=1

69) N1=1, N2=1, N3=1, N4=3, N5=1, and N6=0

70) N1=1, N2=1, N3=1, N4=3, N5=1, and N6=1

71) N1=1, N2=1, N3=1, N4=3, N5=2, and N6=0

72) N1=1, N2=1, N3=1, N4=3, N5=2, and N6=1

73) N1=1, N2=1, N3=1, N4=3, N5=3, and N6=0

74) N1=1, N2=1, N3=1, N4=3, N5=3, and N6=1

75) N1=1, N2=1, N3=1, N4=3, N5=0, and N6=1

76) N1=1, N2=1, N3=1, N4=1, N5=1, and N6=1

77) N1=1, N2=1, N3=1, N4=1, N5=2, and N6=1

78) N1=1, N2=1, N3=1, N4=1, N5=3, and N6=1

79) N1=1, N2=0, N3=0, N4=2, N5=1, and N6=0

80) N1=1, N2=0, N3=0, N4=2, N5=2, and N6=0

81) N1=1, N2=0, N3=0, N4=2, N5=3, and N6=0

82) N1=1, N2=0, N3=0, N4=2, N5=0, and N6=1

83) N1=1, N2=0, N3=0, N4=3, N5=1, and N6=0

84) N1=1, N2=0, N3=0, N4=3, N5=2, and N6=0

85) N1=1, N2=0, N3=0, N4=3, N5=3, and N6=0

86) N1=1, N2=0, N3=0, N4=3, N5=0, and N6=1

87) N1=1, N2=0, N3=0, N4=1, N5=2, and N6=1

88) N1=1, N2=0, N3=0, N4=1, N5=3, and N6=1

89) N1=1, N2=0, N3=0, N4=2, N5=1, and N6=1

90) N1=1, N2=0, N3=0, N4=2, N5=2, and N6=1

91) N1=1, N2=0, N3=0, N4=2, N5=3, and N6=1

92) N1=1, N2=0, N3=0, N4=3, N5=1, and N6=1

93) N1=1, N2=0, N3=0, N4=3, N5=2, and N6=1

94) N1=1, N2=0, N3=0, N4=3, N5=3, and N6=1

95) N1=1, N2=0, N3=0, N4=1, N5=1, and N6=1

96) N1=2, N2=0, N3=0, N4=1, N5=0, and N6=0

97) N1=2, N2=0, N3=1, N4=1, N5=0, and N6=0

98) N1=2, N2=0, N3=0, N4=2, N5=0, and N6=0

99) N1=2, N2=0, N3=0, N4=3, N5=0, and N6=0

100) N1=2, N2=0, N3=0, N4=1, N5=1, and N6=0

101) N1=2, N2=0, N3=0, N4=1, N5=2, and N6=0

102) N1=2, N2=0, N3=0, N4=1, N5=3, and N6=0

103) N1=2, N2=0, N3=0, N4=1, N5=0, and N6=1

104) N1=2, N2=0, N3=1, N4=2, N5=0, and N6=0

105) N1=2, N2=0, N3=1, N4=3, N5=0, and N6=0

106) N1=2, N2=0, N3=1, N4=1, N5=1, and N6=0

107) N1=2, N2=0, N3=1, N4=1, N5=2, and N6=0

108) N1=2, N2=0, N3=1, N4=1, N5=3, and N6=0

109) N1=2, N2=0, N3=1, N4=1, N5=0, and N6=1

110) N1=2, N2=0, N3=1, N4=2, N5=1, and N6=0

111) N1=2, N2=0, N3=1, N4=2, N5=1, and N6=1

112) N1=2, N2=0, N3=1, N4=2, N5=2, and N6=0

113) N1=2, N2=0, N3=1, N4=2, N5=2, and N6=1

114) N1=2, N2=0, N3=1, N4=2, N5=3, and N6=0

115) N1=2, N2=0, N3=1, N4=2, N5=3, and N6=1

116) N1=2, N2=0, N3=1, N4=2, N5=0, and N6=1

117) N1=2, N2=0, N3=1, N4=3, N5=1, and N6=0

118) N1=2, N2=0, N3=1, N4=3, N5=1, and N6=1

119) N1=2, N2=0, N3=1, N4=3, N5=2, and N6=0

120) N1=2, N2=0, N3=1, N4=3, N5=2, and N6=1

121) N1=2, N2=0, N3=1, N4=3, N5=3, and N6=0

122) N1=2, N2=0, N3=1, N4=3, N5=3, and N6=1

123) N1=2, N2=0, N3=1, N4=3, N5=0, and N6=1

124) N1=2, N2=0, N3=1, N4=1, N5=1, and N6=1

125) N1=2, N2=0, N3=1, N4=1, N5=2, and N6=1

126) N1=2, N2=0, N3=1, N4=1, N5=3, and N6=1

127) N1=2, N2=1, N3=0, N4=1, N5=0, and N6=0

128) N1=2, N2=1, N3=0, N4=2, N5=0, and N6=0

129) N1=2, N2=1, N3=0, N4=3, N5=0, and N6=0

130) N1=2, N2=1, N3=0, N4=1, N5=1, and N6=0

131) N1=2, N2=1, N3=0, N4=1, N5=2, and N6=0

132) N1=2, N2=1, N3=0, N4=1, N5=3, and N6=0

133) N1=2, N2=1, N3=0, N4=1, N5=0, and N6=1

134) N1=2, N2=1, N3=0, N4=2, N5=1, and N6=0

135) N1=2, N2=1, N3=0, N4=2, N5=1, and N6=1

136) N1=2, N2=1, N3=0, N4=2, N5=2, and N6=0

137) N1=2, N2=1, N3=0, N4=2, N5=2, and N6=1

138) N1=2, N2=1, N3=0, N4=2, N5=3, and N6=0

139) N1=2, N2=1, N3=0, N4=2, N5=3, and N6=1

140) N1=2, N2=1, N3=0, N4=2, N5=0, and N6=1

141) N1=2, N2=1, N3=0, N4=3, N5=1, and N6=0

142) N1=2, N2=1, N3=0, N4=3, N5=1, and N6=1

143) N1=2, N2=1, N3=0, N4=3, N5=2, and N6=0

144) N1=2, N2=1, N3=0, N4=3, N5=2, and N6=1

145) N1=2, N2=1, N3=0, N4=3, N5=3, and N6=0

146) N1=2, N2=1, N3=0, N4=3, N5=3, and N6=1

147) N1=2, N2=1, N3=0, N4=3, N5=0, and N6=1

148) N1=2, N2=1, N3=0, N4=1, N5=1, and N6=1

149) N1=2, N2=1, N3=0, N4=1, N5=2, and N6=1

150) N1=2, N2=1, N3=0, N4=1, N5=3, and N6=1

151) N1=2, N2=1, N3=1, N4=1, N5=0, and N6=0

152) N1=2, N2=1, N3=1, N4=2, N5=0, and N6=0

153) N1=2, N2=1, N3=1, N4=3, N5=0, and N6=0

154) N1=2, N2=1, N3=1, N4=1, N5=1, and N6=0

155) N1=2, N2=1, N3=1, N4=1, N5=2, and N6=0

156) N1=2, N2=1, N3=1, N4=1, N5=3, and N6=0

157) N1=2, N2=1, N3=1, N4=1, N5=0, and N6=1

158) N1=2, N2=1, N3=1, N4=2, N5=1, and N6=0

159) N1=2, N2=1, N3=1, N4=2, N5=1, and N6=1

160) N1=2, N2=1, N3=1, N4=2, N5=2, and N6=0

161) N1=2, N2=1, N3=1, N4=2, N5=2, and N6=1

162) N1=2, N2=1, N3=1, N4=2, N5=3, and N6=0

163) N1=2, N2=1, N3=1, N4=2, N5=3, and N6=1

164) N1=2, N2=1, N3=1, N4=2, N5=0, and N6=1

165) N1=2, N2=1, N3=1, N4=3, N5=1, and N6=0

166) N1=2, N2=1, N3=1, N4=3, N5=1, and N6=1

167) N1=2, N2=1, N3=1, N4=3, N5=2, and N6=0

168) N1=2, N2=1, N3=1, N4=3, N5=2, and N6=1

169) N1=2, N2=1, N3=1, N4=3, N5=3, and N6=0

170) N1=2, N2=1, N3=1, N4=3, N5=3, and N6=1

171) N1=2, N2=1, N3=1, N4=3, N5=0, and N6=1

172) N1=2, N2=1, N3=1, N4=1, N5=1, and N6=1

173) N1=2, N2=1, N3=1, N4=1, N5=2, and N6=1

174) N1=2, N2=1, N3=1, N4=1, N5=3, and N6=1

175) N1=2, N2=0, N3=0, N4=2, N5=1, and N6=0

176) N1=2, N2=0, N3=0, N4=2, N5=2, and N6=0

177) N1=2, N2=0, N3=0, N4=2, N5=3, and N6=0

178) N1=2, N2=0, N3=0, N4=2, N5=0, and N6=1

179) N1=2, N2=0, N3=0, N4=3, N5=1, and N6=0

180) N1=2, N2=0, N3=0, N4=3, N5=2, and N6=0

181) N1=2, N2=0, N3=0, N4=3, N5=3, and N6=0

182) N1=2, N2=0, N3=0, N4=3, N5=0, and N6=1

183) N1=2, N2=0, N3=0, N4=1, N5=2, and N6=1

184) N1=2, N2=0, N3=0, N4=1, N5=3, and N6=1

185) N1=2, N2=0, N3=0, N4=2, N5=1, and N6=1

186) N1=2, N2=0, N3=0, N4=2, N5=2, and N6=1

187) N1=2, N2=0, N3=0, N4=2, N5=3, and N6=1

188) N1=2, N2=0, N3=0, N4=3, N5=1, and N6=1

189) N1=2, N2=0, N3=0, N4=3, N5=2, and N6=1

190) N1=2, N2=0, N3=0, N4=3, N5=3, and N6=1

191) N1=2, N2=0, N3=0, N4=1, N5=1, and N6=1

192) N1=3, N2=0, N3=0, N4=1, N5=0, and N6=0

193) N1=3, N2=0, N3=1, N4=1, N5=0, and N6=0

194) N1=3, N2=0, N3=0, N4=2, N5=0, and N6=0

195) N1=3, N2=0, N3=0, N4=3, N5=0, and N6=0

196) N1=3, N2=0, N3=0, N4=1, N5=1, and N6=0

197) N1=3, N2=0, N3=0, N4=1, N5=2, and N6=0

198) N1=3, N2=0, N3=0, N4=1, N5=3, and N6=0

199) N1=3, N2=0, N3=0, N4=1, N5=0, and N6=1

200) N1=3, N2=0, N3=1, N4=2, N5=0, and N6=0

201) N1=3, N2=0, N3=1, N4=3, N5=0, and N6=0

202) N1=3, N2=0, N3=1, N4=1, N5=1, and N6=0

203) N1=3, N2=0, N3=1, N4=1, N5=2, and N6=0

204) N1=3, N2=0, N3=1, N4=1, N5=3, and N6=0

205) N1=3, N2=0, N3=1, N4=1, N5=0, and N6=1

206) N1=3, N2=0, N3=1, N4=2, N5=1, and N6=0

207) N1=3, N2=0, N3=1, N4=2, N5=1, and N6=1

208) N1=3, N2=0, N3=1, N4=2, N5=2, and N6=0

209) N1=3, N2=0, N3=1, N4=2, N5=2, and N6=1

210) N1=3, N2=0, N3=1, N4=2, N5=3, and N6=0

211) N1=3, N2=0, N3=1, N4=2, N5=3, and N6=1

212) N1=3, N2=0, N3=1, N4=2, N5=0, and N6=1

213) N1=3, N2=0, N3=1, N4=3, N5=1, and N6=0

214) N1=3, N2=0, N3=1, N4=3, N5=1, and N6=1

215) N1=3, N2=0, N3=1, N4=3, N5=2, and N6=0

216) N1=3, N2=0, N3=1, N4=3, N5=2, and N6=1

217) N1=3, N2=0, N3=1, N4=3, N5=3, and N6=0

218) N1=3, N2=0, N3=1, N4=3, N5=3, and N6=1

219) N1=3, N2=0, N3=1, N4=3, N5=0, and N6=1

220) N1=3, N2=0, N3=1, N4=1, N5=1, and N6=1

221) N1=3, N2=0, N3=1, N4=1, N5=2, and N6=1

222) N1=3, N2=0, N3=1, N4=1, N5=3, and N6=1

223) N1=3, N2=1, N3=0, N4=1, N5=0, and N6=0

224) N1=3, N2=1, N3=0, N4=2, N5=0, and N6=0

225) N1=3, N2=1, N3=0, N4=3, N5=0, and N6=0

226) N1=3, N2=1, N3=0, N4=1, N5=1, and N6=0

227) N1=3, N2=1, N3=0, N4=1, N5=2, and N6=0

228) N1=3, N2=1, N3=0, N4=1, N5=3, and N6=0

229) N1=3, N2=1, N3=0, N4=1, N5=0, and N6=1

230) N1=3, N2=1, N3=0, N4=2, N5=1, and N6=0

231) N1=3, N2=1, N3=0, N4=2, N5=1, and N6=1

232) N1=3, N2=1, N3=0, N4=2, N5=2, and N6=0

233) N1=3, N2=1, N3=0, N4=2, N5=2, and N6=1

234) N1=3, N2=1, N3=0, N4=2, N5=3, and N6=0

235) N1=3, N2=1, N3=0, N4=2, N5=3, and N6=1

236) N1=3, N2=1, N3=0, N4=2, N5=0, and N6=1

237) N1=3, N2=1, N3=0, N4=3, N5=1, and N6=0

238) N1=3, N2=1, N3=0, N4=3, N5=1, and N6=1

239) N1=3, N2=1, N3=0, N4=3, N5=2, and N6=0

240) N1=3, N2=1, N3=0, N4=3, N5=2, and N6=1

241) N1=3, N2=1, N3=0, N4=3, N5=3, and N6=0

242) N1=3, N2=1, N3=0, N4=3, N5=3, and N6=1

243) N1=3, N2=1, N3=0, N4=3, N5=0, and N6=1

244) N1=3, N2=1, N3=0, N4=1, N5=1, and N6=1

245) N1=3, N2=1, N3=0, N4=1, N5=2, and N6=1

246) N1=3, N2=1, N3=0, N4=1, N5=3, and N6=1

247) N1=3, N2=1, N3=1, N4=1, N5=0, and N6=0

248) N1=3, N2=1, N3=1, N4=2, N5=0, and N6=0

249) N1=3, N2=1, N3=1, N4=3, N5=0, and N6=0

250) N1=3, N2=1, N3=1, N4=1, N5=1, and N6=0

251) N1=3, N2=1, N3=1, N4=1, N5=2, and N6=0

252) N1=3, N2=1, N3=1, N4=1, N5=3, and N6=0

253) N1=3, N2=1, N3=1, N4=1, N5=0, and N6=1

254) N1=3, N2=1, N3=1, N4=2, N5=1, and N6=0

255) N1=3, N2=1, N3=1, N4=2, N5=1, and N6=1

256) N1=3, N2=1, N3=1, N4=2, N5=2, and N6=0

257) N1=3, N2=1, N3=1, N4=2, N5=2, and N6=1

258) N1=3, N2=1, N3=1, N4=2, N5=3, and N6=0

259) N1=3, N2=1, N3=1, N4=2, N5=3, and N6=1

260) N1=3, N2=1, N3=1, N4=2, N5=0, and N6=1

261) N1=3, N2=1, N3=1, N4=3, N5=1, and N6=0

262) N1=3, N2=1, N3=1, N4=3, N5=1, and N6=1

263) N1=3, N2=1, N3=1, N4=3, N5=2, and N6=0

264) N1=3, N2=1, N3=1, N4=3, N5=2, and N6=1

265) N1=3, N2=1, N3=1, N4=3, N5=3, and N6=0

266) N1=3, N2=1, N3=1, N4=3, N5=3, and N6=1

267) N1=3, N2=1, N3=1, N4=3, N5=0, and N6=1

268) N1=3, N2=1, N3=1, N4=1, N5=1, and N6=1

269) N1=3, N2=1, N3=1, N4=1, N5=2, and N6=1

270) N1=3, N2=1, N3=1, N4=1, N5=3, and N6=1

271) N1=3, N2=0, N3=0, N4=2, N5=1, and N6=0

272) N1=3, N2=0, N3=0, N4=2, N5=2, and N6=0

273) N1=3, N2=0, N3=0, N4=2, N5=3, and N6=0

274) N1=3, N2=0, N3=0, N4=2, N5=0, and N6=1

275) N1=3, N2=0, N3=0, N4=3, N5=1, and N6=0

276) N1=3, N2=0, N3=0, N4=3, N5=2, and N6=0

277) N1=3, N2=0, N3=0, N4=3, N5=3, and N6=0

278) N1=3, N2=0, N3=0, N4=3, N5=0, and N6=1

279) N1=3, N2=0, N3=0, N4=1, N5=2, and N6=1

280) N1=3, N2=0, N3=0, N4=1, N5=3, and N6=1

281) N1=3, N2=0, N3=0, N4=2, N5=1, and N6=1

282) N1=3, N2=0, N3=0, N4=2, N5=2, and N6=1

283) N1=3, N2=0, N3=0, N4=2, N5=3, and N6=1

284) N1=3, N2=0, N3=0, N4=3, N5=1, and N6=1

285) N1=3, N2=0, N3=0, N4=3, N5=2, and N6=1

286) N1=3, N2=0, N3=0, N4=3, N5=3, and N6=1

287) N1=3, N2=0, N3=0, N4=1, N5=1, and N6=1

288) N1=4, N2=0, N3=0, N4=1, N5=0, and N6=0

289) N1=4, N2=0, N3=1, N4=1, N5=0, and N6=0

290) N1=4, N2=0, N3=0, N4=2, N5=0, and N6=0

291) N1=4, N2=0, N3=0, N4=3, N5=0, and N6=0

292) N1=4, N2=0, N3=0, N4=1, N5=1, and N6=0

293) N1=4, N2=0, N3=0, N4=1, N5=2, and N6=0

294) N1=4, N2=0, N3=0, N4=1, N5=3, and N6=0

295) N1=4, N2=0, N3=0, N4=1, N5=0, and N6=1

296) N1=4, N2=0, N3=1, N4=2, N5=0, and N6=0

297) N1=4, N2=0, N3=1, N4=3, N5=0, and N6=0

298) N1=4, N2=0, N3=1, N4=1, N5=1, and N6=0

299) N1=4, N2=0, N3=1, N4=1, N5=2, and N6=0

300) N1=4, N2=0, N3=1, N4=1, N5=3, and N6=0

301) N1=4, N2=0, N3=1, N4=1, N5=0, and N6=1

302) N1=4, N2=0, N3=1, N4=2, N5=1, and N6=0

303) N1=4, N2=0, N3=1, N4=2, N5=1, and N6=1

304) N1=4, N2=0, N3=1, N4=2, N5=2, and N6=0

305) N1=4, N2=0, N3=1, N4=2, N5=2, and N6=1

306) N1=4, N2=0, N3=1, N4=2, N5=3, and N6=0

307) N1=4, N2=0, N3=1, N4=2, N5=3, and N6=1

308) N1=4, N2=0, N3=1, N4=2, N5=0, and N6=1

309) N1=4, N2=0, N3=1, N4=3, N5=1, and N6=0

310) N1=4, N2=0, N3=1, N4=3, N5=1, and N6=1

311) N1=4, N2=0, N3=1, N4=3, N5=2, and N6=0

312) N1=4, N2=0, N3=1, N4=3, N5=2, and N6=1

313) N1=4, N2=0, N3=1, N4=3, N5=3, and N6=0

314) N1=4, N2=0, N3=1, N4=3, N5=3, and N6=1

315) N1=4, N2=0, N3=1, N4=3, N5=0, and N6=1

316) N1=4, N2=0, N3=1, N4=1, N5=1, and N6=1

317) N1=4, N2=0, N3=1, N4=1, N5=2, and N6=1

318) N1=4, N2=0, N3=1, N4=1, N5=3, and N6=1

319) N1=4, N2=1, N3=0, N4=1, N5=0, and N6=0

320) N1=4, N2=1, N3=0, N4=2, N5=0, and N6=0

321) N1=4, N2=1, N3=0, N4=3, N5=0, and N6=0

322) N1=4, N2=1, N3=0, N4=1, N5=1, and N6=0

323) N1=4, N2=1, N3=0, N4=1, N5=2, and N6=0

324) N1=4, N2=1, N3=0, N4=1, N5=3, and N6=0

325) N1=4, N2=1, N3=0, N4=1, N5=0, and N6=1

326) N1=4, N2=1, N3=0, N4=2, N5=1, and N6=0

327) N1=4, N2=1, N3=0, N4=2, N5=1, and N6=1

328) N1=4, N2=1, N3=0, N4=2, N5=2, and N6=0

329) N1=4, N2=1, N3=0, N4=2, N5=2, and N6=1

330) N1=4, N2=1, N3=0, N4=2, N5=3, and N6=0

331) N1=4, N2=1, N3=0, N4=2, N5=3, and N6=1

332) N1=4, N2=1, N3=0, N4=2, N5=0, and N6=1

333) N1=4, N2=1, N3=0, N4=3, N5=1, and N6=0

334) N1=4, N2=1, N3=0, N4=3, N5=1, and N6=1

335) N1=4, N2=1, N3=0, N4=3, N5=2, and N6=0

336) N1=4, N2=1, N3=0, N4=3, N5=2, and N6=1

337) N1=4, N2=1, N3=0, N4=3, N5=3, and N6=0

338) N1=4, N2=1, N3=0, N4=3, N5=3, and N6=1

339) N1=4, N2=1, N3=0, N4=3, N5=0, and N6=1

340) N1=4, N2=1, N3=0, N4=1, N5=1, and N6=1

341) N1=4, N2=1, N3=0, N4=1, N5=2, and N6=1

342) N1=4, N2=1, N3=0, N4=1, N5=3, and N6=1

343) N1=4, N2=1, N3=1, N4=1, N5=0, and N6=0

344) N1=4, N2=1, N3=1, N4=2, N5=0, and N6=0

345) N1=4, N2=1, N3=1, N4=3, N5=0, and N6=0

346) N1=4, N2=1, N3=1, N4=1, N5=1, and N6=0

347) N1=4, N2=1, N3=1, N4=1, N5=2, and N6=0

348) N1=4, N2=1, N3=1, N4=1, N5=3, and N6=0

349) N1=4, N2=1, N3=1, N4=1, N5=0, and N6=1

350) N1=4, N2=1, N3=1, N4=2, N5=1, and N6=0

351) N1=4, N2=1, N3=1, N4=2, N5=1, and N6=1

352) N1=4, N2=1, N3=1, N4=2, N5=2, and N6=0

353) N1=4, N2=1, N3=1, N4=2, N5=2, and N6=1

354) N1=4, N2=1, N3=1, N4=2, N5=3, and N6=0

355) N1=4, N2=1, N3=1, N4=2, N5=3, and N6=1

356) N1=4, N2=1, N3=1, N4=2, N5=0, and N6=1

357) N1=4, N2=1, N3=1, N4=3, N5=1, and N6=0

358) N1=4, N2=1, N3=1, N4=3, N5=1, and N6=1

359) N1=4, N2=1, N3=1, N4=3, N5=2, and N6=0

360) N1=4, N2=1, N3=1, N4=3, N5=2, and N6=1

361) N1=4, N2=1, N3=1, N4=3, N5=3, and N6=0

362) N1=4, N2=1, N3=1, N4=3, N5=3, and N6=1

363) N1=4, N2=1, N3=1, N4=3, N5=0, and N6=1

364) N1=4, N2=1, N3=1, N4=1, N5=1, and N6=1

365) N1=4, N2=1, N3=1, N4=1, N5=2, and N6=1

366) N1=4, N2=1, N3=1, N4=1, N5=3, and N6=1

367) N1=4, N2=0, N3=0, N4=2, N5=1, and N6=0

368) N1=4, N2=0, N3=0, N4=2, N5=2, and N6=0

369) N1=4, N2=0, N3=0, N4=2, N5=3, and N6=0

370) N1=4, N2=0, N3=0, N4=2, N5=0, and N6=1

371) N1=4, N2=0, N3=0, N4=3, N5=1, and N6=0

372) N1=4, N2=0, N3=0, N4=3, N5=2, and N6=0

373) N1=4, N2=0, N3=0, N4=3, N5=3, and N6=0

374) N1=4, N2=0, N3=0, N4=3, N5=0, and N6=1

375) N1=4, N2=0, N3=0, N4=1, N5=2, and N6=1

376) N1=4, N2=0, N3=0, N4=1, N5=3, and N6=1

377) N1=4, N2=0, N3=0, N4=2, N5=1, and N6=1

378) N1=4, N2=0, N3=0, N4=2, N5=2, and N6=1

379) N1=4, N2=0, N3=0, N4=2, N5=3, and N6=1

380) N1=4, N2=0, N3=0, N4=3, N5=1, and N6=1

381) N1=4, N2=0, N3=0, N4=3, N5=2, and N6=1

382) N1=4, N2=0, N3=0, N4=3, N5=3, and N6=1

383) N1=4, N2=0, N3=0, N4=1, N5=1, and N6=1

The different structures that can comprise the components are described in other sections of this document that detail components for ET.

In a preferred embodiment N1 = 1 and N4= 1.

In a preferred embodiment, The targeting ligands are selective for receptors increased on tumor cells and the efector agents are drugs that exert synergistic toxicity. In a preferred embodiment the effector groups E1...En exert synergystic toxicity by inhibiting the denovo synthesis of vital cellular factors and also inhibit salvage pathways related to these factors. In a preferred embodiment, at least one component of the set of effector groups En functions outside the cells and inhibits salvage pathways.

In a preferred embodiment, E1...En inhibit the denovo synthesis of purine and or pyrimidine metabolites and related uptake and salvage pathways. In preferred embodiments, E1...En inhibit denovo synthesis by inhibiting one or more of the following enzymes:

1.) thymidylate synthase
2.) ribonucleotide reductase
3.) glycinamide ribonucleotide transformylase
4.) 5-aminoimidazole-4-carboxamide ribonucleotide transferase
5.) dihydroorotate dehydrogenase
6.) carbamoyl phosphate synthetase
7.) orotidine-5'-phosphate decarboxylase
8.) inosine 5'monophosphate dehydrogenase
9.) aspartate transcarbamylase

and inhibit salvage pathways by inhibiting one or more of the following :

1.) nucleoside transporter proteins
2.) thymidine kinase
3.) uridine/cytidine kinase

4.) deoxycytidine kinase

5.) deoxyguanosine kinase

6.) hypoxanthine-guanine phosphoribosyltransferase

7.) xanthine-guanine phosphoribosyltransferase

8.) adenine phosphoribosyltransferase

In a preferred embodiment, the targeted set of denovo and salvage pathway inhibitors are used in conjunction with a non-targeted analog related to the inhibited pathways that can be taken up by cells even in the presence of the salvage pathway inhibitors; and wherein the non-targeted inhibitor provides additional synergystic toxicity.

In a preferred embodiment, the set E1...En can inhibit both thymidine monophosphate synthesis and thymidine transport, and azidothymidine (AZT) is administered concurrently in a non-targeted manner.

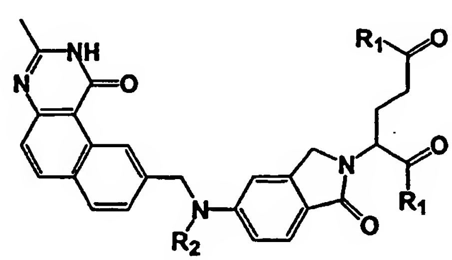
AZT, which enters cells by a mechanism independent of the nucleoside transporter system is known to inhibit thymidine kinase and potentiate the toxicity of inhibitors of denovo thymidine synthesis. The following references relate to this subject matter: Weber G., et al., "Regulation of De Novo and Salvage Pathways in Chemotherapy," *Adv Enzyme Regul,* 31:45-67 (1991); Weber G., et al., "Salvage Capacity of Hepatoma 3924A and Action of Dipyridamole," *Adv Enzyme Regul,* 21:53-69 (1983); Zimmerman T.P., et al., "3'-azido-3'-deoxythymidine. An Unusual Nucleoside Analogue that Permeates the Membrane of Human Erythrocytes and Lymphocytes by Nonfacilitated Diffusion," *J Biol Chem,* 262(12):5748-54 (1987); Chan T.C.K., et al.,

"Permeation and Metabolism of Anti-HIV and Endogenous Nucleosides in Human Immune Effector Cells," *Biochemical Pharmacology,* 46(2):273-278 (1993); Betageri G.V., et al., "Effect of Dipyridamole on Transport and Phosphorylation of Thymidine and 3'-azido-3'-deoxythymidine in Human Monocyte/Macrophages," *Biochemical Pharmacology,* 404:867-870 (1990); Van Mouwerik T.J., et al., "Augmentation of Methotrexate Cytotoxicity in Human Colon Cancer Cells Achieved Through Inhibition of Thymidine Salvage by Dipyridamole," *Biochemical Pharmacology,* 36(6):809-814 (1987); Andreuccetti M., et al., "Azidothymidine in Combination with 5-fluorouracil in Human Colorectal Cell Lines: In Vitro Synergistic Cytotoxicity and DNS-Induced Strand-Breaks," *Eur J Cancer,* 32A(7):1219-26 (1996); Zhen Y.S., et al., "Azidothymidine and Dipyridamole as Biochemical Response Modifiers: Synergism with Methotrexate and 5-Fluorouracil in Human Colon and Pancreatic Carcinoma Cells," *Oncol Res,* 4(2):73-8 (1992); Lehman N.L.; Danenberg P.V., "Modulation of RTX Cytotoxicity by Thymidine and Dipyridamole in Vitro: Implications for Chemotherapy," *Cancer Chemother Pharmacol,* 45(2):142-8 (2000); Smith P.G., et al., "Dipyridamole Potentiates the in Vitro Activity of MTA (LY231514) by Inhibition of Thymidine Transport," *Br J Cancer,* 82(4):924-30 (2000); Weber G., et al., "AZT: A Biochemical Response Modifier of Methotrexate and 5-Fluorouracil Cytotoxicity in Human Ovarian and Pancreatic Carcinoma Cells," *Cancer Commun,* 3(4):127-32 (1991); Weber G., et al., "Azidothymidine Inhibition of Thymidine Kinase and Synergistic Cytotoxicity with Methotrexate and 5-Fluorouracil in Rat Hepatoma and Human Colon Cancer Cells," *Cancer Commun,* 2(4):129-33 (1990); Zimmerman T.P., et al., "Inhibition of Thymidine Transport by 3'-azido-3'-deoxythymidine and its Metabolites,"

Oncol Res, 5(12):483-7 (1993), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention consists of the set of targeted drugs E1-T1 and E2-T2, wherein E1 comprises an inhibitor to thymidylate synthase and E2 comprises an inhibitor to nucleoside transporters. A preferred embodiment of E1 is based on the compound 1843U89 which is an extremely potent inhibitor of thymidylate synthase with a Ki of 90 pM. The following references relate to this subject matter: Duch D.S., et al., "Biochemical and Cellular Pharmacology of 1843U89, a Novel Benzoquinazoline Inhibitor of Thymidylate Synthase," *Cancer Res,* 53(4):810-8 (1993); Stout T.J.; Stroud R.M., "The complex of the Anti-Cancer Therapeutic, BW1843U89, with Thymidylate Synthase at 2.0 a Resolution: Implications for a New Mode of Inhibition," *Structure,* 4(1):67-77 (1996), the contents of which are incorporated herein by reference in their entirety.

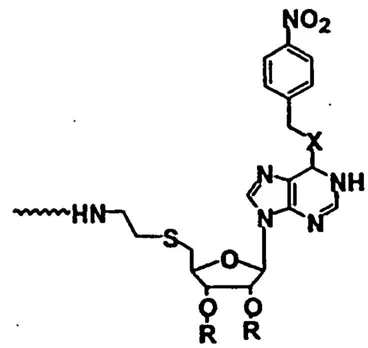
In a preferred embodiment E1 comprises the following structure referred to as



1E1.1:

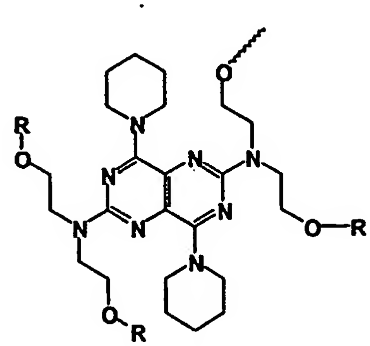
wherein R_1 is OH or the site of attachment of a linker or trigger connected to the remainder of the drug complex, and R_2 is H or the site of attachment of a trigger; and wherein the trigger, when activated, cleaves the R_1-C bond or the R_2-N bond.

And, E2 comprises the structure below referred to as embodiment 1E2.1 :



wherein R is H or a bioreversible hydroxy masking group that undergoes spontaneous or enzymatically triggered cleavage to expose the free hydroxy moiety; and wherein the wavy line is the site of linker attachment to the remainder of the drug; and wherein X is NH or S. The masking group can allow the targeting group T2 of the drug rather than E2 to define the targeting specificity. The principle is exactly the same as described previously for the case of masked intracellular transporter ligands.

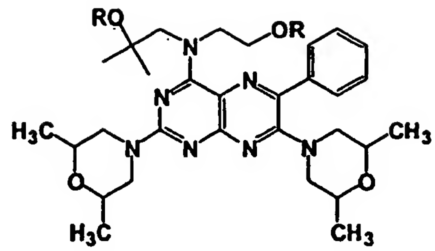
Or E2 comprises the structure below referred to as embodiment 1E2.2:



wherein the wavy line is the site of linker attachment to the remainder of the drug complex, and R is H or a bioreversible hydroxy masking group or masking trigger that undergoes spontaneous or enzymatically triggered cleavage to expose the free hydroxy moiety.

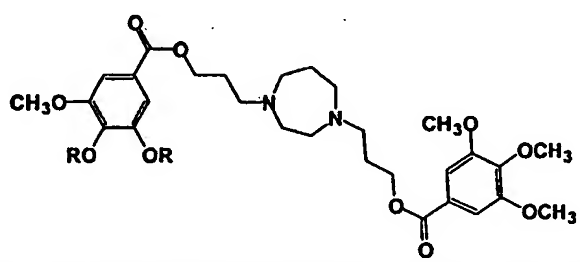
This structure is based upon the ability of dipyridamole to block nucleoside transporter function. The masking group can be employed to allow the targeting group T2 of the drug rather than E2 to define the targeting specificity. In addition, the masking group can prevent the tight binding of the dipyridamole moiety to acidic glycoprotein. It can be emphasized that targeting can tether the dipyridamole group to the cell surface and result in extremely high effective concentrations at the nucleoside transporter sites at the site of action on the cell surface. The following references relate to this subject matter: Baldwin S.A., et al., "Nucleoside Transporters: Molecular Biology and Implications for Therapeutic Development," *Molecular Med Today,* 5:216-224 (1999); Bamford C.H., et al., "Polymeric Inhibitors of Platelet Aggregation. II. Copolymers of Dipyridamole and Related Drugs with *N*-vinylpyrrolidone," *Biochimica et Biophysica Acta,* 924:38-44 (1987), the contents of which are incorporated herein by reference in their entirety.

Or E2 comprises the structure below referred to as embodiment 1E2.3:



wherein R is H or a bioreversible hydroxy masking group that undergoes spontaneous or enzymatically triggered cleavage to expose the free hydroxy moiety; or wherein R is the site of linker attachment to the remainder of ET. This structure is based upon the ability of compound BIBW 22 to inhibit nucleoside transport. The following references relate to this subject matter: Chen H., et al., "BIBW 22, a Dipyridamole Analogue, Acts as a Bifunctional Modulator on Tumor Cells by Influencing Both P-Glycoprotein and Nucleoside Transport," *Cancer Research,* 53:1974-1977 (1993), the contents of which are incorporated herein by reference in their entirety.

Or E2 comprises the following structure (referred to as embodiment 1E2.4),



which is based upon the ability of dilazep to inhibit nucleoside transport.

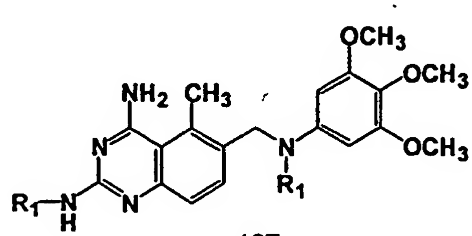
Wherein R is H or a bioreversible hydroxy masking group that undergoes spontaneous or enzymatically triggered cleavage to expose the free hydroxy moiety or wherein R is the site of linker attachment to the remainder of ET.

Another preferred embodiment is based on the super synergystic toxicity that results from the combination of folic acid, inhibitors of dihydrofolate reductase, and inhibitors of other folate dependent enzymes such as glycinamide ribonucleotide formyltransferase, 5-aminomidazole-4-carboxamide ribonucleotide formyltransferase, and thymidylate synthase. The mechanisms responsible for this super synergystic toxicity are poorly understood. The following references relate to this subject matter: Gaumont Y., et al., "Quantitation of Folic Acid Enhancement of Antifolate Synergism," *Cancer Research,* 52:2228-2235 (1992); Faessel H.M., et al., "Super *in Vitro* Synergy between Inhibitors of Dihydrofolate Reductase and Inhibitors of other Folate-requiring Enzymes: The Critical Role of Polyglutamylation," *Cancer Research,* 58:3036-3050 (1998); Kisliuk R.L., et al., "The Effect of Polyglutamylation on the Inhibitory Activity of Folate Analogs," *In: D.Goldman (ed.), Proceedings of the Second Workshop on Folyl and Antifolyl Polyglutamates,* pp. 319-328. New York: Praeger (1985); Kisliuk R.L., et al., "Synergistic Growth Inhibition by Combination of Antifolates," *In: M.F. Picciano, et al., (eds.), Evaluation of Folate Metabolism in Health and Disease,* pp. 79-89, New York: Alan R. Liss (1990); Galivan J., et al., "Antifolate Drug Interactions: Enhancement of Growth Inhibition Due to the Antipurine 5,10-Dideazatetrahydrofolic Acid by the Lipophilic Dihydrofolate Reductase Inhibitors Metoprine and Trimetrexate," *Cancer Res,* 48:2421-2425 (1988); Galivan J., et al., "Synergistic Growth

Inhibition of Rat Hepatoma Cells Exposed *in Vitro* to N^{10}-Propargyl-5,8-dideazafolate with Methotrexate or the Lipophilic Antifolates Trimetrexate or Metoprine," *Cancer Res,* 47:5256-5260 (1987); Faessel H.M., et al., "Folic Acid-enhanced Synergy for the Combination of Trimetrexate Plus the Glycinamide Ribonucleotide Formyltransferase Inhibitor 4-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimidino[5,4,6][1,4]thiazin-6-yl)-(S)-ethyl]-2,5-thienoylamino-L-glutamic Acid (AG2034): Comparison Across Sensitive and Resistant Human Tumor Cell Lines," *Biochem Pharmacol,* 57(5):567-77 (1999); Galivan J., et al., "The Role of Cellular Folates in the Enhancement of Activity of the Thymidylate Synthase Inhibitor 10-Propargyl-5,8-dideazafolate against Hepatoma Cells *in Vitro* by Inhibitors of Dihydrofolate Reductase," *J Biological Chem,* 264(18):10685-10692 (1989), the contents of which are incorporated herein by reference in their entirety.

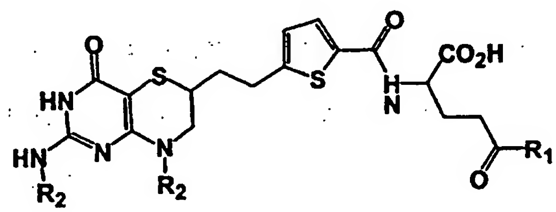
A preferred embodiment of the present invention consists of the set of targeted drugs E1-T1 and E2-T2, wherein E1 comprises an inhibitor of dihydrofolate reductase and E2 comprises an inhibitor of glycinamide ribonucleotide formyltransferase, 5-aminomidazole-4-carboxamide ribonucleotide formyltransferase, or thymidylate synthase, and folic acid is administer in conjunction with the targeted drugs E1-T1 and E2-T2.

In preferred embodiments, E1 comprises the structure (2E1.1) shown below:



wherein R_1 is H, or a bioreversible amino masking group which when triggered by enzymatic or spontaneous processes cleaves the R_1-N bond and wherein R_1 can also bear a site of linker attachment to the remainder of the drug complex. Activation of the trigger can liberate the dihydrofolate reductase inhibitor trimetrexate.

and E2 comprises the structure (2E2.1) shown below:

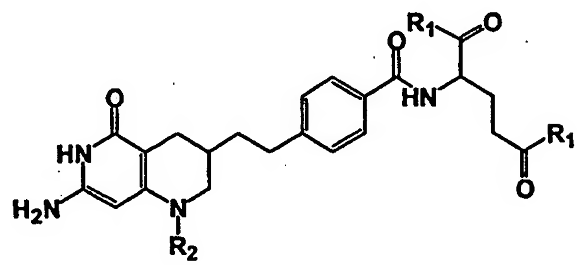


wherein R_1 is OH, or the site of linker attachment to the remainder of the drug complex, and R_2 is H, or a bioreversible amino protecting group which when triggered by enzymatic or spontaneous mechanisms unmasks the free amino group, and where R2 can also bear a site of linker attachment to the remainder of ET complex.

The above structure is based on AG2034 a compound that is a potent inhibitor of glycinamide ribonucleotide formyltransferase. The masking group R_2 can be used to prevent binding to the folate receptor from defining the domain of targeting specificity. The following references relate to this subject matter: Varney M.D., et al., "Protein Structure-Based Design, Synthesis, and Biological Evaluation of 5-Thia-2,6-diamino-4(*3H*)-oxopyrimidines: Potent Inhibitors of Glycinamide Ribonucleotide Transformylase with Potent Cell Growth Inhibition," *J Med Chem,* 40:2502-2524 (1997); Boritzki T.J., et al., "AG2034: A Novel

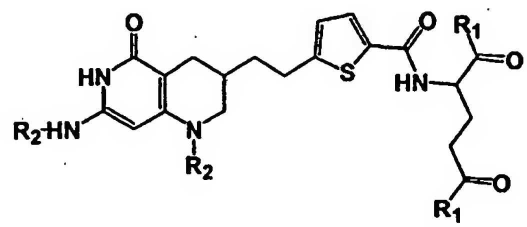
Inhibitor of Glycinamide Ribonucleotide Formyltransferase," *Invest New Drugs,* 14(3):295-303 (1996), the contents of which are incorporated herein by reference in their entirety.

Or E2 comprises the structure (2E2.2) shown below:



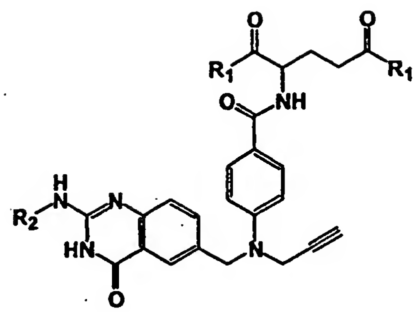
wherein R_1 is OH, or the site of linker attachment to the remainder of the drug complex; or wherein R_1 can be a bioreversible protecting group which when triggered unmasks the carboxylate group and to which is attached a linker connnected to the remainder of the drug complex, and R_2 is H, or a bioreversible amino protecting group which when triggered by enzymatic or spontaneous mechanisms unmasks the free amino group. This structure is based on Lometrexol a compound that is a potent inhibitor of glycinamide ribonucleotide formyltransferase. The following references relate to this subject matter: Roberts J.D., et al., "Weekly Lometrexol with Daily Oral Folic Acid is Appropriate for Phase II Evaluation," *Cancer Chemother Pharmacol,* 45(2):103-10 (2000), the contents of which are incorporated herein by reference in their entirety.

Or E2 comprises the structure (2E2.3) shown below:



wherein R_1 is OH, or the site of linker attachment to the remainder of the drug complex; or wherein R_1 can be a bioreversible protecting group which when triggered unmasks the carboxylate group and to which is attached a linker connected to the remainder of the drug complex, and R_2 is H, or a bioreversible amino protecting group which when triggered by enzymatic or spontaneous mechanisms unmasks the free amino group; and wherein R_2 can have a site of linker attachment to the remainder of ET. This structure is based on LY309887 a compound that is a potent inhibitor of glycinamide ribonucleotide formyltransferase. The following references relate to this subject matter: Mendelsohn L.G., et al., "Biochemistry and Pharmacology of Glycinamide Ribonucleotide Formyltransferase Inhibitors: LY309887 and Lometrexol," *Invest New Drugs,* 14(3):287-94 (1996), the contents of which are incorporated herein by reference in their entirety.

Or E2 comprises the structure (2E2.3) shown below::



wherein R_1 is OH, or the site of linker attachment to the remainder of the drug complex; or wherein R_1 can be a bioreversible protecting group which when triggered unmasks the carboxylate group and to which is attached a linker connnected to the remainder of the drug complex, and R_2 is H, or a bioreversible amino protecting group which when triggered by enzymatic or spontaneous mechanisms unmasks the free amino group; and wherein R_2 can have a site of linker attachment to the remainder of the drug complex. This structure is based on 10-propargyl-5-8-dideazafolic acid, a potent inhibitor of thymidylate synthase.

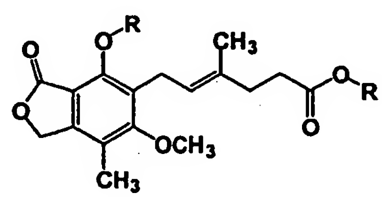
In a preferred embodiment the above are E1-T1 and E2-T2 are administered in conjunction with folic acid.

Another preferred embodiment is based on the synergystic toxicity that results from the inhibition of denovo guanine nucleotide synthesis and the salvage pathway by inhibition of hypoxanthine-guanine phosphoribosyltransferase. The following references relate to this subject matter: Weber G., et al., "Regulation of De Novo and Salvage Pathways in Chemotherapy," *Adv Enzyme Regul,* 31:45-67 (1991); Weber G., et al., "Salvage Capacity of Hepatoma 3924A and Action of Dipyridamole", *Adv Enzyme Regul,* 21:53-69 (1983); Digits J.A.; Hedstrom L.,

"Species-Specific Inhibition of Inosine 5'-Monophosphate Dehydrogenase by Mycophenolic Acid," *Biochemistry,* 38:15388-15397 (1999), the contents of which are incorporated herein by reference in their entirety.

A preferred embodiment of the present invention consists of the set of targeted drugs E1-T1 and E2-T2, wherein E1 comprises an inhibitor to inosine monophosphate dehydrogenase and E2 comprises an inhibitor to hypoxanthine-guanine phosphoribosyltransferase.

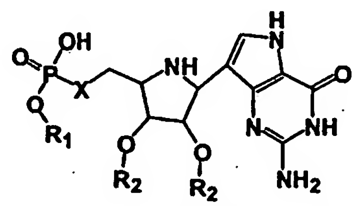
In a preferred embodiment, E1 comprises the following structure referred to embodiment 3E1.1:



wherein R is H, or a bioreversible masking group which when triggered by enzymatic or chemical processes exposes the free OH group, and wherein R can have a site of linker attachment to the remainder of the drug complex. This structure is based on mycophenolic acid, which inhibits IMP dehydrogenase at nanomolar levels. The following references relate to this subject matter: Shi W., et al., "The 2.0 Å Structure of Human Hypoxanthine-guanine Phosphoribosyltransferase in Complex with a Transition-state Analog Inhibitor," *Nature Structural Biology,* 6(6):588-593 (1999); Digits J.A.; Hedstrom L., "Species-Specific Inhibition of Inosine 5'-Monophosphate Dehydrogenase by

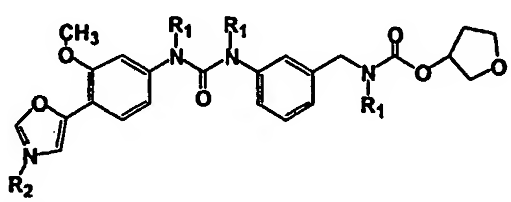
Mycophenolic Acid," *Biochemistry,* 38:15388-15397 (1999), the contents of which are incorporated herein by reference in their entirety.

And, E2 comprises the structure referred to as 3E2.1:



wherein R_1 and R_2 is H, or a bioreversible masking group which when triggered by enzymatic or chemical processes exposes the free OH group; and wherein R_1 or R_2 can can have a site of linker attachment to the remainder of ET. This structure is based on immucillin GP, which inhibits hypoxanthine-guanine phosphoribosyltransferase at low nonmolar levels. The following references relate to this subject matter: Shi W., et al., "The 2.0 Å Structure of Human Hypoxanthine-guanine Phosphoribosyltransferase in Complex with a Transition-state Analog Inhibitor," *Nature Structural Biology,* 6(6):588-593 (1999), the contents of which are incorporated herein by reference in their entirety.

Or E1 can comprise the following structure (3E1.2):



wherein R_1 is H or a bioreversible masking group which when triggered cleaves the R-N bond, and wherein R_1 or R_2 can have a site of linker attachment to the remainder of ET; and wherein R_2 can be a bioreversible masking group which

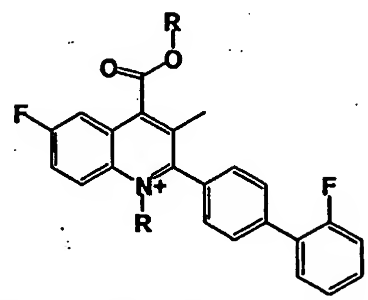
when triggered cleaves the R-N bond, or wherein R_2 can be absent from the structure. Activation of the trigger can release VX-497, which is a potent inhibitor of IMP dehydrogenase. The following references relate to this subject matter: Markland W., et al., "Broad-Spectrum Antiviral Activity of the IMP Dehydrogenase Inhibitor VX-497: a Comparison with Ribavirin and Demonstration of Antiviral Additivity with Alpha Interferon," *Antimicrobial Agents and Chemotherapy,* 44(4):859-866 (2000), the contents of which are incorporated herein by reference in their entirety.

Additional synergystic toxicity would be expected upon the addition of a third drug E3-T3 in which E3 is a nucleoside transport inhibitor as described above as embodiments 1E2.1, 1E2.2, 1E2.3 and 1E2.4.

A preferred embodiment of the present invention consists of the set of targeted drugs E1-T1 and E2-T2, wherein E1 comprises an inhibitor to dihydroorotic acid dehydrogenase and E2 comprises an inhibitor to nucleoside transport. Dihydroorotic acid dehydrogenase is the fourth enzyme in the committed pathway of de novo pyrimidine synthesis. A preferred embodiment is based on brequinar, a compound that inhibits dihydroorotic acid dehydrogenase at nonamolar levels. The following references relate to this subject matter: Bruneau J.M., et al., "Purification of Human Dihydro-orotate Dehydrogenase and its Inhibition by A77 1726, The Active Metabolite of Leflunomide," *Biochem J,* 336, 299-303 (1998); Chen S.F., et al., "Inhibition of Dihydroorotate Dehydrogenase Activity by Brequinar Sodium," *Cancer Res,* 52:3521-3527

(1992), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E1 comprises the following structure (4E1.1):



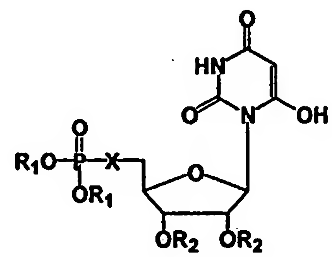
wherein R is H or the site of bioreversible masking group to which is attached a linker connected to the remainder of the drug complex, wherein spontaneous or enzymatic triggering unmasks the the active enzyme inhibitor.

And, E2 is comprised of an inhibitor to nucleoside transport such as described previously in embodiments 1E2.1, 1E2.2, 1E2.3 and 1E2.4

A preferred embodiment of the present invention consists of the set of targeted drugs E1-T1 and E2-T2, wherein E1 comprises an inhibitor to orotidine 5'-phosphate decarboxylase and E2 comprises an inhibitor to nucleoside transport. Orotidine 5'-phosphate decarboxylase catalyzes the final step in the de novo synthesis of uridine monophosphate. A preferred embodiment is based upon 1-(5'-phospho- -ribofuranosyl)barbituric acid which is a potent inhibitor of the enzyme. The following references relate to this subject matter: Levine H.L., et al., "Inhibition of Orotidine-5'-phosphate Decarboxylase by 1-(5'-Phospho-β-D-

ribofuranosyl)barbituric Acid, 6-Azauridine 5'-Phosphate, and Uridine 5'-Phosphate," *Biochemistry,* 19:4993-4999 (1980), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E1 comprises the following structure (5E1.1):



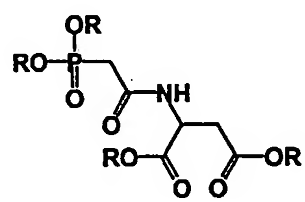
wherein in X is O, CH_2, or S, and R_1 is H, or a bioreversible phosphate protecting group which when triggered by spontaneous or enzymatic processes unmasks the free phosphate; and in which R_1 can have a site of linker attachment to the remainder of ET; and wherein R_2 is H or a bioreversible hydroxy protecting group which when activated unmasks the free hydroxy group and wherein R_2 can have a site of linker attachment to the remainder of ET.

And, E2 is comprised of an inhibitor to nucleoside transport such as described previously in embodiments 1E2.1, 1E2.2, 1E2.3 and 1E2.4

In a preferred embodiment, E1 comprises an inhibitor of aspartate transcarbamylase, the second key enzyme in the de novo synthesis of pyrimidine rings. A preferred embodiment is based on (N-phosphonoacetyl)-L-aspartate, which is a potent inhibitor of aspartate transcarbamylase. The following references relate to this subject matter: Erlichman C., "An Overview of

the Clinical Pharmacology of N-phosphonacetyl-L-aspartate (PALA), a New Antimetabolite," *Recent Results Cancer Res,* 74:65-71 (1980); Johnson R.K., et al., "Antitumor Activity of N-(phosphonacetyl)-L-aspartic Acid, a Transition-State Inhibitor of Aspartate Transcarbamylase," *Cancer Res,* 36(8):2720-5 (1976); Erlichman C.; Vidgen D., "Antitumor Activity of N-phosphonacetyl-L-aspartic Acid in Combination with Nitrobenzylthioinosine," *Biochem Pharmacol,* 33(20):3177-81 (1984), the contents of which are incorporated herein by reference in their entirety.

In a preferred embodiment, E1 comprises the following structure (6E1.1):



Wherein R is H, or a bioreversible protecting group which when triggered by spontaneous or enzymatic processes unmasks the free phosphonate or carboxylate group, and in which R can have a site of linker attachment to the remainder of ET.

And, E2 is comprised of an inhibitor to nucleoside transport such as described previously in embodiments 1E2.1, 1E2.2, 1E2.3 and 1E2.4

Methods of Use

The compounds of the present invention are used by contacting the target cells with a sufficient quantity to evoke the desired diagnostic or therapeutic result. The drugs can be administered in combination with commonly employed pharmacological excipients, preservatives and stabilizers that are well known to one skilled in the arts. In general, the drugs are for intravenous use and can be administered dissolved in sterile saline or water or a buffered salt solution. In selected situations the drugs could be given routes such as intra-arterially, intra-peritoneally, orally or topically.

The drugs should be administered to a patient in a sufficient amount and for a sufficient period of time to achieve the desired pharmacological result and will depend upon the severity of the illness and the other factor well known to one skilled in the art. For a drug ET in which E is comprised of a known drug, the dose of ET can be lower than or about equal to the dose of drug E as currently used in clinical practice. The dose of the drug administered can be in the range of about 1 picogram per kilogram body weight to about 50 mg/kg.

In a preferred embodiment the drugs ET are administered at ultra-low dose as described below. In other embodiments the drug ET is given at conventional doses similar to those currently used for the drug E. Procedures for dose optimization are well known to one skilled in the art.

For diagnostic use, routine procedures and methodologies applicable to the detection and imaging of the targeted moiety can be used.

Anti-cancer Therapy

Targeted Toxins

The following general guidelines and principles are relevant to the use of anti-cancer drugs of the class described herein.

1.) The smallest dose of drug that exceeds that required to saturate the target receptors on the tumor cells can be used. This is referred to, in this patent application, as "ultra-low dose". This can be a dose that results in subnanomolar to picomolar plasma concentrations or can be higher depending upon the affinity of the particular drug for the tumor cells. The use of excess drug dosage can lower the targeting selectivity and therapeutic index without increasing therapeutic efficacy.

2.) Only a subset of tumor cells at any given time can be able to contact the drug. Tumors are heterogeneous with respect to drug penetration even for small molecules. Accordingly, multiple cycles of therapy can be used.

3.) Only a subset of the tumor cells can be sensitive to any particular drug. There is no point to single agent therapy. Accordingly, multiple drugs can be used concurrently. The extremely low doses employed can allow for the simultaneous administration of effective doses of multiple targeted agents without prohibitive toxicity.

4.) When applicable, the drugs can be used in conjunction with agents that suppress delivery to non-tumor areas. For example, a drug, which includes a targeting ligand against glutamate carboxypeptidase II for the

treatment of prostate or breast cancer, can be used in conjunction with an orally administered nonabsorbable inhibitor to the enzyme to suppress targeting to the enzyme on the luminal surface of the small intestine.

5.) If a particular targeted drug has significant nonspecific affinity to serum proteins then it is advisable to administer a pharmaceutical agent, which competitively displaces the targeted drug (displacer drug) from the serum protein. Conceptually, this is similar to the displacement of phenytoin by salicylate from serum albumen. Since the displacer drug can be selected to be of very low toxicity, concentrations thousands of times higher then the target drug can be employed to give effective competitive inhibition of the nonspecific protein binding.

6.) The targeted toxin class of drugs can be used in conjunction with targeted drugs that stimulate the innate or adaptive immune system. These drugs can provoke an inflammatory reaction at the tumor site. These targeted drugs can be given first, and then after about 48 hours when a tumor inflammatory reaction is present, the targeted toxin type drugs can be administered. The inflammatory reaction can facilitate the tumor penetration of the drugs. Targeted toxin type drugs may also be given concurrently with targeted immunostimulator type drugs.

7.) If the drug has a detoxifying trigger that is activated by an independently targeted antibody enzyme conjugate then the drug can be administered after the detoxifying enzyme has localized to the non-tumor cells.

8.) If the drug has a tumor-selective trigger, that can be activated by an enzyme independently targeted to tumor cells, then the drug can be administered first, allowed to localize to target cells, and then the targeted enzyme trigger administered.

9.) If the drug bears a masked transporter ligand comprised of masked biotin then the drug can be administered, allowed to localize to target cells, and then the avidin-transporter moieties can be administered.

10.) The drugs can be used in addition to other anti-cancer therapeutic modalities such as surgery, radiation therapy, angiogenisis inhibitors, and immunotherapy.

11.) Tumor cells develop resistance to drugs by predictable "escape mechanisms". The typical response of tumors to a metabolic inhibitor is a compensatory increase in the expression of the targeted enzyme and increased expression of enzymes that by-pass the inhibited metabolic step. By the administration of a combination of drugs these "escape mechanisms" can be transformed into an ever-tightening noose which amplifies tumor killing. The use of a metabolic inhibitor, coupled with targeting, directed against the mechanisms of resistance to that inhibitor,

can increase tumor killing cells by the very mechanisms that typically confer drug resistance.

Ultra-low Dose Multiple Drug Multiple Target Therapy

It is increasingly apparent that cancer is not a single disease, but a changing spectrum of different diseases even in an individual patient. The average colon cancer cell has over ten thousand different DNA mutations. The following references relate to this subject matter: Stoler D.L., et al., "The Onset and Extent of Genomic Instability in Sporadic Colorectal Tumor Progression," *PNAS,* 96(26):15121-15126 (1999), the contents of which are incorporated herein by reference in their entirety.

A patient with disseminated cancer can have 1 trillion (10^{12}) cancer cells spread throughout the body. To ensure eradication of the disease, it is necessary to kill every last cancer cell without undo toxicity to the patient. Any single drug against any single tumor target can give at most a 2 to 4 log reduction in tumor cell burden, which represents killing of 99% to 99.99% of the tumor cells. Potentially any one of the residual tumor cells can grow and cause progressive illness from cancer. The only way to deal with this is to use multiple independent drugs or therapies directed against multiple tumor targets. If the probability that a tumor cell can develop resistance to a single drug is 10^{-2} then the joint probability that a tumor cell could develop resistance simultaneously to 10 independent drugs is 10^{-20}. In other words, the combination of ten independent drugs can be a billion billion times more effective than a single drug.

Today it is difficult to treat cancer patients with even one or two anti-cancer drugs at a time, because the drugs are poorly selective and highly toxic. A severe price is paid if a patient's tumor is resistant to the anti-cancer drugs. Toxicity often precludes the administration of effective doses of alternate drugs. What is needed is a failure tolerant anti-cancer technology based on the reality that no single drug can be effective. What is needed is a technology to enable the use of multiple drugs against multiple targets so that the probability of tumor escape is precluded.

The technology detailed in this patent application is designed to enable the simultaneous administration of multiple drugs against multiple tumor targets without undo toxicity. The high binding affinity and selectivity that multifunctional drug delivery vehicles can have for tumor cells can translate into the ability to effectively target tumor cells with ultra-low nontoxic doses of drugs.

The present invention is a method of treating cancer that is comprised of the administration of ultra-low doses of multiple drugs targeted against multiple properties of the tumor. The definition of "ultra-low dose" was previously given. The drugs can be given simultaneously or in sufficient temporal proximity that for resistance to develop, the tumor cells must acquire joint resistance to each agent. The method of ultra-low dose multiple drug multiple target therapy is by its very design inherently failure tolerant. The key is redundancy of targeting and mechanisms of tumor cell killing. In this method the average tumor cell can be exposed to numerous (about 2 to about 20) different drugs, any single one of which would be sufficient to kill the tumor cell. Although this can seem like

massive over-kill reminiscent of the nuclear arms race, this is what is realistically needed to address the problem of cancer. Killing the average cancer cell is clinically meaningless. What is needed is to kill the last cancer cell. Tumor heterogeneity mandates the use of multiple drugs against multiple targets to achieve this goal.

Extremely minute quantities of anti-cancer drugs when delivered into cancer cells can be lethal to the cell. For example, 500 molecules of bleomycin delivered intracellularly are sufficient to kill the cell. The following references relate to this subject matter: Pron G., et al., "Internalisation of the Bleomycin Molecules Responsible for Bleomycin Toxicity: A Receptor-mediated Endocytosis Mechanism," *Biochemical Pharmacology*, 57:45-56 (1999), the contents of which are incorporated herein by reference in their entirety.

In principal, given ideal drug delivery, a patient with widely disseminated cancer and a tumor burden of 100 billion cancer cells could be treated with 5×10^{13} molecules or approximately 10^{-10} moles of drug. For a 100 kg patient this represents a drug concentration of about 10^{-12} Molar. The drugs embodied by the present invention are designed to approach this ideal, but in practice unachievable, theoretical limit of minimal drug dose. Currently in actual practice patients are treated with bleomycin at doses approximately 100,000 times higher.

The method of ultra-low dose multiple drug multiple target therapy is based upon the ability of multifunctional drug delivery vehicles to selectively deliver and trap

cytotoxic concentrations of drug inside tumor cells at doses far below levels which can produce systemic toxicity. The tighter the binding affinity the lower the drug concentration that is required to saturate the target receptors on the tumor cells and deliver a lethal dose of drug to the tumor cells. The drugs embodied by the present invention are expected to bind effectively to tumor cells at concentrations that are orders of magnitude lower than the levels needed to produce systemic toxicity.

A preferred embodiment consists of administering to a patient with cancer the drugs (E1-T1), (E2-T2) and ...(En-Tn); which are compounds of the present invention; and wherein the drugs are directed against or selective for multiple sets of targets that are increased on tumor cells; and wherein the drugs deliver multiple different antitumor agents. Preferably the delivered effector agents should be such that tumor resistance develops by different independent mechanisms for each drug. The drugs are administered systemically for a sufficient duration, at a sufficient dose, and sufficient frequency to achieve the desired antitumor response.

In a preferred embodiment of the above, the doses are ultra-low wherein ultra-low refers to a minimal dose that is sufficient to bind the drug to target receptors on accessible tumor cells. An accessible tumor cell is a tumor cell that is able to contact the drug.

Targeted Masked Antigens and Targeted Neoantigens

Drugs, which exert activity by evoking an immune response to a targeted masked antigen or a targeted neoantigen, require that the patient be pre-sensitized to the relevant antigens prior to drug therapy. This can be accomplished by immunizing the patient with the respective unmasked antigen or neoantigen in combination with a variety of adjuvants and immunostimulators. The antigen can be administered by a variety of routes with the intradermal route being preferred. Only that portion of the drug bearing the antigenic moiety or the neoantigen is used for immunization purposes. In some cases, it can be desirable to use an antigenic moiety with a short linker bearing a reactive group such as an isothiocyanate group. The function of this group is to increase the immunogenicity of the antigen by enhancing uptake and presentation by dendritic cells. As discussed previously, the sensitization can also be conducted in vitro and adoptively transferred by the infusion of sensitized lymphocytes.

The drugs of the present invention may be given to either a person or an animal in need of the pharmaceutical effect of said drugs.

Additional Preferred Embodiments of the Invention:

Preferred Combinations of Tumor Targeting Ligands

In preferred embodiments ;

designated: (embodiment TLP #.X, wherein X is the number given below to the pairs of target receptors and X=1,2,3,... 795);

ET is an anti-cancer drug or diagnostic drug comprised of one targeting ligand that binds the first target receptor (a1) and a second targeting ligand that binds to the second target receptor (a2) indicated in the pairs of (a1 — a2) listed below:

1) urokinase — a cathepsin type protease;
2) urokinase — a collagenase;
3) urokinase — a gelatinase;
4) urokinase — a matrix metalloproteinase;
5) urokinase — a membrane type matrix metalloproteinase;
6) urokinase — alpha v beta 3 integrin;
7) urokinase — bombesin /gastrin releasing peptide receptors;
8) urokinase — cathepsin B;
9) urokinase — cathepsin D;
10) urokinase — to cathepsin K;
11) urokinase — cathepsin L;
12) urokinase — cathepsin O;
13) urokinase — fibroblast activation protein;
14) urokinase — folate binding receptors;

15) urokinase — gastrin/cholecystokinin type B receptor;

16) urokinase — glutamate carboxypeptidase II or (PSMA);

17) urokinase — guanidinobenzoatase;

18) urokinase — laminin receptor;

19) urokinase — matrilysin;

20) urokinase — matripase;

21) urokinase — melanocyte stimulating hormone receptor;

22) urokinase — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

23) urokinase — norepinephrine transporters;

24) urokinase — nucleoside transporter proteins;

25) urokinase — peripheral benzodiazepam binding receptors;

26) urokinase — plasmin;

27) urokinase — seprase;

28) urokinase — sigma receptors;

29) urokinase — somatostatin receptors;

30) urokinase — stromelysin 3;

31) urokinase — trypsin;

32) urokinase — urokinase;

33) urokinase — MMP 1;

34) urokinase — MMP 2;

35) urokinase — MMP 3;

36) urokinase — MMP 7;

37) urokinase — MMP 9;

38) urokinase — membrane type matrix metalloproteinase I;

39) urokinase — MMP 12;

40) urokinase — MMP 13;

41) urokinase — a tumor antigen;

42) plasmin — a cathepsin type protease;

43) plasmin — a collagenase;

44) plasmin — a gelatinase;

45) plasmin — a matrix metalloproteinase;

46) plasmin — a membrane type matrix metalloproteinase;

47) plasmin — alpha v beta 3 integrin;

48) plasmin — bombesin /gastrin releasing peptide receptors;

49) plasmin — cathepsin B;

50) plasmin — cathepsin D;

51) plasmin — to cathepsin K;

52) plasmin — cathepsin L;

53) plasmin — cathepsin O;

54) plasmin — fibroblast activation protein;

55) plasmin — folate binding receptors;

56) plasmin — gastrin/cholecystokinin type B receptor;

57) plasmin — glutamate carboxypeptidase II or (PSMA);

58) plasmin — guanidinobenzoatase;

59) plasmin — laminin receptor;

60) plasmin — matrilysin;

61) plasmin — matripase;

62) plasmin — melanocyte stimulating hormone receptor;

63) plasmin — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

64) plasmin — norepinephrine transporters;

65) plasmin — nucleoside transporter proteins;

66) plasmin — peripheral benzodiazepam binding receptors;

67) plasmin — plasmin;

68) plasmin — seprase;

69) plasmin — sigma receptors;

70) plasmin — somatostatin receptors;

71) plasmin — stromelysin 3;

72) plasmin — trypsin;

73) plasmin — urokinase;

74) plasmin — MMP 1;

75) plasmin — MMP 2;

76) plasmin — MMP 3;

77) plasmin — MMP 7;

78) plasmin — MMP 9;

79) plasmin — membrane type matrix metalloproteinase I;

80) plasmin — MMP 12;

81) plasmin — MMP 13;

82) plasmin — a tumor antigen;

83) a collagenase — a cathepsin type protease;

84) a collagenase — a collagenase;

85) a collagenase — a gelatinase;

86) a collagenase — a matrix metalloproteinase;

87) a collagenase — a membrane type matrix metalloproteinase;

88) a collagenase — alpha v beta 3 integrin;

89) a collagenase — bombesin /gastrin releasing peptide receptors;

90) a collagenase — cathepsin B;

91) a collagenase — cathepsin D;

92) a collagenase — to cathepsin K;

93) a collagenase — cathepsin L;

94) a collagenase — cathepsin O;

95) a collagenase — fibroblast activation protein;

96) a collagenase — folate binding receptors;

97) a collagenase — gastrin/cholecystokinin type B receptor;

98) a collagenase — glutamate carboxypeptidase II or (PSMA);

99) a collagenase — guanidinobenzoatase;

100) a collagenase — laminin receptor;

101) a collagenase — matrilysin;

102) a collagenase — matripase;

103) a collagenase — melanocyte stimulating hormone receptor;

104) a collagenase — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

105) a collagenase — norepinephrine transporters;

106) a collagenase — nucleoside transporter proteins;

107) a collagenase — peripheral benzodiazepam binding receptors;

108) a collagenase — seprase;

109) a collagenase — sigma receptors;

110) a collagenase — somatostatin receptors;

111) a collagenase — stromelysin 3;

112) a collagenase — trypsin;

113) a collagenase — a collagenase;

114) a collagenase — MMP 1;

115) a collagenase — MMP 2;

116) a collagenase — MMP 3;

117) a collagenase — MMP 7;

118) a collagenase — MMP 9;

119) a collagenase — membrane type matrix metalloproteinase I;

120) a collagenase — MMP 12;

121) a collagenase — MMP 13;

122) a collagenase — a tumor antigen;

123) a gelatinase — a cathepsin type protease;

124) a gelatinase — a gelatinase;

125) a gelatinase — a matrix metalloproteinase;

126) a gelatinase — a membrane type matrix metalloproteinase;

127) a gelatinase — alpha v beta 3 integrin;

128) a gelatinase — bombesin /gastrin releasing peptide receptors;

129) a gelatinase — cathepsin B;

130) a gelatinase — cathepsin D;

131) a gelatinase — to cathepsin K;

132) a gelatinase — cathepsin L;

133) a gelatinase — cathepsin O;

134) a gelatinase — fibroblast activation protein;

135) a gelatinase — folate binding receptors;

136) a gelatinase — gastrin/cholecystokinin type B receptor;

137) a gelatinase — glutamate carboxypeptidase II or (PSMA);

138) a gelatinase — guanidinobenzoatase;

139) a gelatinase — laminin receptor;

140) a gelatinase — matrilysin;

141) a gelatinase — matripase;

142) a gelatinase — melanocyte stimulating hormone receptor;

143) a gelatinase — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

144) a gelatinase — norepinephrine transporters;

145) a gelatinase — nucleoside transporter proteins;

146) a gelatinase — peripheral benzodiazepam binding receptors;

147) a gelatinase — seprase;

148) a gelatinase — sigma receptors;

149) a gelatinase — somatostatin receptors;

150) a gelatinase — stromelysin 3;

151) a gelatinase — trypsin;

152) a gelatinase — MMP 1;

153) a gelatinase — MMP 2;

154) a gelatinase — MMP 3;

155) a gelatinase — MMP 7;

156) a gelatinase — MMP 9;

157) a gelatinase — membrane type matrix metalloproteinase I;

158) a gelatinase — MMP 12;

159) a gelatinase — MMP 13;

160) a gelatinase — a tumor antigen;

161) a matrix metalloproteinase — a cathepsin type protease;

162) a matrix metalloproteinase — a collagenase;

163) a matrix metalloproteinase — a gelatinase;

164) a matrix metalloproteinase — a matrix metalloproteinase;

165) a matrix metalloproteinase — a membrane type matrix metalloproteinase;

166) a matrix metalloproteinase — alpha v beta 3 integrin;

167) a matrix metalloproteinase — bombesin /gastrin releasing peptide receptors;

168) a matrix metalloproteinase — cathepsin B;

169) a matrix metalloproteinase — cathepsin D;

170) a matrix metalloproteinase — to cathepsin K;

171) a matrix metalloproteinase — cathepsin L;

172) a matrix metalloproteinase — cathepsin O;

173) a matrix metalloproteinase — fibroblast activation protein;

174) a matrix metalloproteinase — folate binding receptors;

175) a matrix metalloproteinase — gastrin/cholecystokinin type B receptor;

176) a matrix metalloproteinase — glutamate carboxypeptidase II or (PSMA);

177) a matrix metalloproteinase — guanidinobenzoatase;

178) a matrix metalloproteinase — laminin receptor;

179) a matrix metalloproteinase — matrilysin;

180) a matrix metalloproteinase — matripase;

181) a matrix metalloproteinase — melanocyte stimulating hormone receptor;

182) a matrix metalloproteinase — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

183) a matrix metalloproteinase — norepinephrine transporters;

184) a matrix metalloproteinase — nucleoside transporter proteins;

185) a matrix metalloproteinase — peripheral benzodiazepam binding receptors;

186) a matrix metalloproteinase — plasmin;

187) a matrix metalloproteinase — seprase;

188) a matrix metalloproteinase — sigma receptors;

189) a matrix metalloproteinase — somatostatin receptors;

190) a matrix metalloproteinase — stromelysin 3;

191) a matrix metalloproteinase — trypsin;

192) a matrix metalloproteinase — a matrix metalloproteinase;

193) a matrix metalloproteinase — MMP 1;

194) a matrix metalloproteinase — MMP 2;

195) a matrix metalloproteinase — MMP 3;

196) a matrix metalloproteinase — MMP 7;

197) a matrix metalloproteinase — MMP 9;

198) a matrix metalloproteinase — membrane type matrix metalloproteinase I;

199) a matrix metalloproteinase — MMP 12;

200) a matrix metalloproteinase — MMP 13;

201) a matrix metalloproteinase — a tumor antigen;

202) a membrane type metalloproteinase — a cathepsin type protease;

203) a membrane type metalloproteinase — a membrane type matrix metalloproteinase;

204) a membrane type metalloproteinase — alpha v beta 3 integrin;

205) a membrane type metalloproteinase — bombesin /gastrin releasing peptide receptors;

206) a membrane type metalloproteinase — cathepsin B;

207) a membrane type metalloproteinase — cathepsin D;

208) a membrane type metalloproteinase — to cathepsin K;

209) a membrane type metalloproteinase — cathepsin L;

210) a membrane type metalloproteinase — cathepsin O;

211) a membrane type metalloproteinase — fibroblast activation protein;

212) a membrane type metalloproteinase — folate binding receptors;

213) a membrane type metalloproteinase — gastrin/cholecystokinin type B receptor;

214) a membrane type metalloproteinase — glutamate carboxypeptidase II or (PSMA);

215) a membrane type metalloproteinase — guanidinobenzoatase;

216) a membrane type metalloproteinase — laminin receptor;

217) a membrane type metalloproteinase — matrilysin;

218) a membrane type metalloproteinase — matripase;

219) a membrane type metalloproteinase — melanocyte stimulating hormone receptor;

220) a membrane type metalloproteinase — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

221) a membrane type metalloproteinase — norepinephrine transporters;

222) a membrane type metalloproteinase — nucleoside transporter proteins;

223) a membrane type metalloproteinase — peripheral benzodiazepam binding receptors;

224) a membrane type metalloproteinase — seprase;

225) a membrane type metalloproteinase — sigma receptors;

226) a membrane type metalloproteinase — somatostatin receptors;

227) a membrane type metalloproteinase — stromelysin 3;

228) a membrane type metalloproteinase — trypsin;

229) a membrane type metalloproteinase — MMP 1;

230) a membrane type metalloproteinase — MMP 2;

231) a membrane type metalloproteinase — MMP 3;

232) a membrane type metalloproteinase — MMP 7;

233) a membrane type metalloproteinase — MMP 9;

234) a membrane type metalloproteinase — membrane type matrix metalloproteinase I;

235) a membrane type metalloproteinase — MMP 12;

236) a membrane type metalloproteinase — MMP 13;

237) a membrane type metalloproteinase — a tumor antigen;

238) alpha v beta 3 integrin — a cathepsin type protease;

239) alpha v beta 3 integrin — alpha v beta 3 integrin;

240) alpha v beta 3 integrin — bombesin /gastrin releasing peptide receptors;

241) alpha v beta 3 integrin — cathepsin B;

242) alpha v beta 3 integrin — cathepsin D;

243) alpha v beta 3 integrin — cathepsin K;

244) alpha v beta 3 integrin — cathepsin L;

245) alpha v beta 3 integrin — cathepsin O;

246) alpha v beta 3 integrin — fibroblast activation protein;

247) alpha v beta 3 integrin — folate binding receptors;

248) alpha v beta 3 integrin — gastrin/cholecystokinin type B receptor;

249) alpha v beta 3 integrin — glutamate carboxypeptidase II or (PSMA);

250) alpha v beta 3 integrin — guanidinobenzoatase;

251) alpha v beta 3 integrin — laminin receptor;

252) alpha v beta 3 integrin — matrilysin;

253) alpha v beta 3 integrin — matripase;

254) alpha v beta 3 integrin — melanocyte stimulating hormone receptor;

255) alpha v beta 3 integrin — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

256) alpha v beta 3 integrin — norepinephrine transporters;

257) alpha v beta 3 integrin — nucleoside transporter proteins;

258) alpha v beta 3 integrin — peripheral benzodiazepam binding receptors;

259) alpha v beta 3 integrin — seprase;

260) alpha v beta 3 integrin — sigma receptors;

261) alpha v beta 3 integrin — somatostatin receptors;

262) alpha v beta 3 integrin — stromelysin 3;

263) alpha v beta 3 integrin — trypsin;

264) alpha v beta 3 integrin — MMP 1;

265) alpha v beta 3 integrin — MMP 2;

266) alpha v beta 3 integrin — MMP 3;

267) alpha v beta 3 integrin — MMP 7;

268) alpha v beta 3 integrin — MMP 9;

269) alpha v beta 3 integrin — membrane type matrix metalloproteinase I;

270) alpha v beta 3 integrin — MMP 12;

271) alpha v beta 3 integrin — MMP 13;

272) alpha v beta 3 integrin — a tumor antigen;

273) cathepsin B — a cathepsin type protease;

274) cathepsin B — bombesin /gastrin releasing peptide receptors;

275) cathepsin B — cathepsin B;

276) cathepsin B — cathepsin D;

277) cathepsin B — to cathepsin K;

278) cathepsin B — cathepsin L;

279) cathepsin B — cathepsin O;

280) cathepsin B — fibroblast activation protein;

281) cathepsin B — folate binding receptors;

282) cathepsin B — gastrin/cholecystokinin type B receptor;

283) cathepsin B — glutamate carboxypeptidase II or (PSMA);

284) cathepsin B — guanidinobenzoatase;

285) cathepsin B — laminin receptor;

286) cathepsin B — matrilysin;

287) cathepsin B — matripase;

288) cathepsin B — melanocyte stimulating hormone receptor;

289) cathepsin B — nitrobenzylthioinosine-binding receptors or (nucleoside transporter);

290) cathepsin B — norepinephrine transporters;

291) cathepsin B — nucleoside transporter proteins;

292) cathepsin B — peripheral benzodiazepam binding receptors;

293) cathepsin B — seprase;

294) cathepsin B — sigma receptors;

295) cathepsin B — somatostatin receptors;

296) cathepsin B — stromelysin 3;

297) cathepsin B — trypsin;

298) cathepsin B — MMP 1;

299) cathepsin B — MMP 2;

300) cathepsin B — MMP 3;

301) cathepsin B — MMP 7;

302) cathepsin B — MMP 9;

303) cathepsin B — membrane type matrix metalloproteinase I;

304) cathepsin B — MMP 12;

305) cathepsin B — MMP 13;

306) cathepsin B — a tumor antigen;